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A Study of Penicillin Ointments and Creams*

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A number of ointments and cream formulas containing penicillin were prepared and studied for their antibactericidal activity against organisms. Tests were made by a new modified agar cup-plate method which was devised in the laboratories of the School of Pharmacy at Purdue University.

INTRODUCTION

IN A PAPER dealing with the topical forms for the application of penicillin Lesser (1) stated that the greatest use of it by the general practitioner would be in the form of ointments and creams. Much smaller concentrations of penicillin are needed for local applications than are required for parenteral use.

The advantages of the topical application are several, such as (*a*) economy, (*b*) greater concentration at the site of infection, (*c*) the effect of a single dose may be greatly prolonged, (*d*) the response to treatment is more prompt, and (*e*) the drug is held in contact with the tissues longer when in the form of an ointment than when in aqueous solution. Lesser stated further that when the two basic problems, namely, the most suitable base and a more stable form of penicillin, were solved greater use of penicillin locally would be made.

In the preparation of a penicillin ointment Burns (2) stated that the prime objective was to insure therapeutic activity and that the base used should not only be smooth but as nearly neutral as possible. Such a preparation should also withstand sterilization without much separation and re-emulsify easily upon cooling. Penicillin ointments should be kept as cool as possible.

Greey and Hebb (3) studied nine different penicillin ointment formulas. The most stable formula was composed of stearic acid, fatty acid esters of sorbitan, and about 77.5 per cent of water.

Mentis (4) obtained excellent results in the treatment of impetigo on infants with a penicillin ointment composed of 500 units per gram of penicillin in Abbott's hydrosorb base. On the other hand, Templeton (5)

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and others tested several ointments of penicillin, by the agar cup-plate method, using aquaphor as the base, and little or no antibacterial activity was indicated. Reports such as these would make it seem that the character of the base makes a difference in the activity of penicillin when used topically.

Payne (6) used a penicillin ointment, composed of 200 units per gram in a base of Lanette Wax 5x with 30 per cent water. He observed that if this was prepared in a copper pan it lost all of its antibacterial activity within three days but if prepared in a glass container the activity was more prolonged.

Dahl (7) reported a number of bases which were satisfactory for use with penicillin. Differences in the retention of sodium penicillin by various bases was noted. Calcium penicillin was preferable to sodium penicillin for ointments. The potency of penicillin ointments was longest in anhydrous bases.

The Massachusetts College of Pharmacy (8) studied the problem of ointment bases for penicillin. Bases of the oil-in-water type were found to be the best vehicles especially when the water content was as much as 70 per cent.

The statement has been made that for the treatment of burns and other superficial wounds, as well as for various skin infections, it is desirable to apply penicillin in a semisolid base. A base composed of equal parts of Lanette wax, soft petrolatum, and water has been proposed as being suitable (9). Lanette wax is a British product not readily available here but substitutes for it have been proposed.

EXPERIMENTAL

The requirements of a satisfactory base for the topical application of penicillin have been reviewed. The object of this research has been to study various ointment and cream bases with penicillin incorporated in them in an effort to develop a formula in which the activity of penicillin could be maintained over an extended period of time under refrigeration.

In the course of this study a modification of the agar cup-plate method was developed (10). The new method is simple and unique. It makes use of a heating element in the formation, in a sterile manner, of uniform agar cups. This is accomplished by standing flat-end Pyrex glass rods, 10 mm. in length and any desired diameter, in liquid agar. The agar is allowed to harden and each of the glass rods is then heated by means of a small electric heating element which, in the form of a loop, is slipped down over the exposed upper end of the rod. The rod, upon becoming warm, melts a surrounding film of the agar in which it stands, and is removed. The agar which melted at the base of the rod forms an agar seal at the bottom of the cup.

Four such cups were made in each Petri dish. Into one cup was placed about 0.50 Gm. of the control composed usually of the unmedicated base. Ointments in 0.50-Gm. amounts and in varying concentrations of penicillin were placed in the other three cups. Next 5 cc. of inoculated agar was poured over the top of the base layer and allowed to cool. The inoculated agar consisted of 2 cc. of a sixteen- to eighteen-hour beef broth culture of Staphylococcus aureus in 100 cc. of melted agar. The Petri dish was then placed in an incubation oven in an inverted position and kept at 37° for sixteen to eighteen hours.

The clear zones around the cups were very uniform and varied in diameter according to the concentration of the penicillin in the ointment. Figure 1 shows the heating unit being used to remove one of the glass rods. Figure 2 shows the seeded agar plate after being incubated. The clear zones are an indication of the antibacterial activity of the penicillin ointment.

FIGURE 1.

Preparation and Testing of Penicillin Ointments.—In the course of this study many ointment formulas were prepared and tested. Only three of them are reported in this paper. The formulas for these bases will be given, following which will be

tables summarizing the antibacterial activity of the ointments as indicated by a modified agar cup-plate method. Inasmuch as these formulas are pharmaceutical preparations which should be compounded *secundum artem*, the procedures for preparing them will be omitted. The data given in the tables are largely self-explanatory.

Sodium penicillin was dissolved in a minimum amount of water and then incorporated into the bases. When those ointments which contained the penicillin in therapeutic concentrations were tested the plates showed no growth of the organisms.

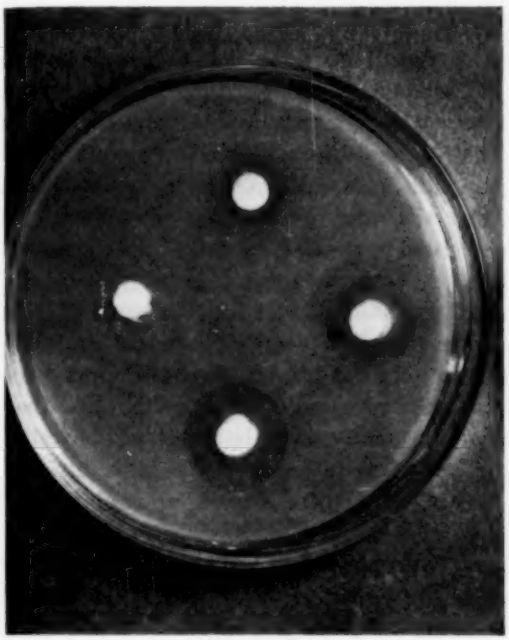

FIGURE 2.

Therefore it was necessary to make tests upon ointments with a very low concentration of penicillin. The concentrations used were as follows: (*a*) one unit per gram, (*b*) one-half unit per gram, (*c*) one-fourth unit per gram, (*d*) control—the base. Portions of about 30 Gm. of each of the above ointments were made and tested at the time prepared and at intervals of several days. This was to observe the loss of antibacterial potency with aging. The ointments were refrigerated during the test period.

FORMULA 1

Cetyl alcohol	4.50 Gm.
Sodium lauryl sulfate	0.50 Gm.
Stearyl alcohol	12.90 Gm.
White petrolatum	17.40 Gm.
Cetaceum	17.40 Gm.
Tween 20	2.30 Gm.
Sterile distilled water	45.00 Gm.

This base was used to prepare the three dilute ointments of penicillin as described above. Their antibacterial activity upon an eighteen to twenty-four hour culture of S. aureus, 209 P. is indicated in Table I. The plain base was used as the control except when otherwise indicated by the agar cup-plate method.

TABLE I

Dates	Ointments with Varying Concentrations of Penicillin, Units/Gm.	Antibacterial Activity Indicated by Clear Zones Measured in Mm. Plates 1	2	3	4
9-22-45	Control[a]	42	43	44	44
9-27-45	Control	0	0	0	0
10-3-45	Control	0	0	0	0
9-22-45	1	26.5	26	27.5	28
9-27-45	1	22	22.2	22	22
10-3-45	1	16	16	15	16
9-22-45	1/2	22	21.5	22	21.5
9-27-45	1/2	13	..	13	11
9-22-45	1/4	20	21	19	21
10-3-45	1/4	12.25	10.5	11	11

[a] The control in this instance was an ointment composed of 250 units of penicillin per gram and illustrates the fact that it is not practicable to test ointments of high penicillin concentration.

Comments.—Two observations may be made from the results indicated in Table I. The first is that the width of the clear zones is in direct proportion to the concentration of penicillin in the ointment. The second is that the activity of the penicillin in the ointment decreased with age even though stored at a low temperature.

FORMULA 2

Stearic acid	20.0 Gm.
Span 60	14.4 Gm.
Span 80	2.0 Gm.
Tween 20	5.6 Gm.
Sterile distilled water	138.0 Gm.

This formula resulted in a soft cream and was suggested by Greey and Hebb (3). The activity of penicillin in this base was tested after the manner described above. The results are given in Table II.

TABLE II

Dates	Creams with Varying Concentrations of Penicillin, Units/Gm.	Antibacterial Activity Indicated by Clear Zones Measured in Mm. Plates 1	2	3	4
8-24-45	Control	0	0	0	0
9-5-45	Control	0	0	0	0
9-8-45	Control	0	0	0	0
9-22-45	Control (250 units)	37	36	37	36
8-24-45	1	26.5	27.5	24.0	23.5
9-5-45	1	26.0	26.0	25.5	24.0
9-8-45	1	26.5	27.5	24.0	23.5
9-22-45	1	17.0	17.0	16.5	18.5
8-24-45	1/2	23.5	24.5	22.0	21.0
9-5-45	1/2	23.5	24.0	24.0	24.0
9-8-45	1/2	23.5	24.5	22.0	21.0
9-22-45	1/2	16.0	16.5	16.0	15.5
8-24-45	1/4	20.0	20.0	18.0	17.0
9-5-45	1/4	22.5	24.0	22.0	24.0
9-8-45	1/4	20.0	20.0	18.0	17.0
9-22-45	1/4	10.0	9	10.0	9.0

Comments.—It should be observed that the control which consisted of the base only gave no evidence of any antibacterial action. The control to which 250 units of penicillin was added showed marked activity. The creams with the 1/4 unit of penicillin per gram showed less antibacterial activity than the stronger creams. All creams maintained activity for about sixteen days. By the thirtieth day all had shown a decided loss in bacterial activity. The ointments were stored at refrigerator temperature while being studied.

FORMULA 3

Cetyl alcohol	21.0 Gm.
Cetaceum	12.1 Gm.
White petrolatum	21.0 Gm.
Sodium lauryl sulfate	0.3 Gm.
Sodium citrate	3.0 Gm.
Urea	4.9 Gm.
Sterile distilled water	37.7 Gm.

This proved to be a satisfactory base. It was used to prepare penicillin ointments of the same concentrations as those reported above, which were stored at refrigerator temperature while being tested.

The antibacterial activity of the ointments prepared from this base are given in Table III.

TABLE III

Dates	Ointments with Varying Concentrations of Penicillin, Units/Gm.	Antibacterial Activity Indicated by Clear Zones Measured in Mm. Plates 1	2	3	4
9-14-45	Control	0	0	0	0
9-27-45	Control	0	0	0	0
9-14-45	1	19.50	19.75	19.50	20.50
9-27-45	1	20.00	20.50	20.25	19.75
9-14-45	1/2	16.50	16.50	17.90	16.50
9-27-45	1/2	16.50	16.00	15.50	16.25
9-14-45	1/4	13.00	13.50	13.00	12.50
9-27-45	1/4	12.50	12.00	12.75	14.00

Comments.—These ointments were tested but twice, thirteen days apart, and showed very little deterioration as to antibacterial activity during that time. A more prolonged study of the activity and stability of penicillin in this base should be made.

Other Formulas.—In addition to these three formulas we studied, in the same manner, the antibacterial activity of penicillin in (*a*) Simple ointment U. S. P. XI, (*b*) Hydrosorb, (*c*) Aquaphor, and (*d*) Aquaphor with 50% water incorporated. The test results were all negative indicating that these bases were not satisfactory for penicillin in low concentrations.

The penicillin was extracted from the Aquaphor-water ointment and tested. The results indicated that the penicillin had not been destroyed in the ointment but was rendered inactive in the base in the concentrations used.

SUMMARY AND CONCLUSIONS

Many ointments and creams containing penicillin in concentrations of one unit, one-half unit, and one-fourth unit per gram have been tested. The most satisfactory formulas were those of the oil-in-water type emulsions containing some of the newer wetting agents. The presence of water in concentrations as high as 70 per cent in these bases, if kept at refrigerator temperatures, was not detrimental to penicillin. In fact those of high water content were the most satisfactory.

The new modified technique which we have proposed for the agar cup-plate test makes it possible to evaluate the activity of penicillin without first having to extract it from the ointment. The availability of penicillin in ointments may be determined by observing the zones of inhibition on the agar plates.

The penicillin was protected in the ointments by the addition of small amounts of sodium citrate and urea. It was fouud that the ointment could be sterilized by the method offered by Payne (6).

This study has been made upon ointments and creams with very low concentrations of penicillin, which deteriorated rather rapidly at room temperature. However, ointments with high or therapeutic concentrations retained their antibacterial activity even after considerable exposure to room temperature.

Finally we believe that this study indicates that a method of assay for penicillin in ointments and creams, by dilution after the manner of testing solutions, is possible. This would necessitate the development of a standard base.

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A Simple Pipette Washer for the Average Laboratory*

By JOHN H. BREWER

MANY DEVICES have been suggested for washing pipettes and there are several available on the open market. For the most part they have too great capacity and are too expensive for the average laboratory. Most of them operate as an automatic fill and drain siphon. The design herein described works on a similar principle but is unique in that it is one piece and may be used with any 1000-cc. graduate or cylinder which has a depth of about 16 inches. It differs from other designs in that it is not necessary to have a hole in the cylinder or container below the top rim or to have the water in the vessel rise above the siphon tube in order to start the siphon. This pipette washer also differs from the usual design, such as that described by Wadsworth (1) in that the water enters the cylinder through the same tube through which it is later discharged.

The siphon (Fig. 1) is very easily constructed of glass in the laboratory, although one made of metal or plastic has been found to give better service in that it is not readily broken. Tubing with a $^5/_{16}$-inch inside diameter has been found satisfactory and may be bent as shown. In use a piece of glass cloth or wire gauze should be placed in the bottom of the cylinder, or graduate, to prevent breaking of the pipette tips and to insure proper washing.

Connect rubber tubing to siphon at point *A* (see diagram) and to water supply. Place tube *B* inside of cylinder containing pipettes to be washed and adjust water flow so that a liter graduate fills in approximately five minutes. The pipette washer will then operate flushing and refilling continuously. The washing operation should be conducted in a laboratory sink or on a drainboard.

Irregularities in water pressure may cause the cylinder to overflow. If this occurs and the water pressure cannot be controlled, it is recommended that a pressure regulating valve, set for one pound, be placed in the line.

Should it be necessary to wash a larger number of pipettes than can be held in a liter graduate, a Pyrex cylinder, six inches in diameter, is available and a stainless steel rack has been constructed to facilitate handling of the pipettes.

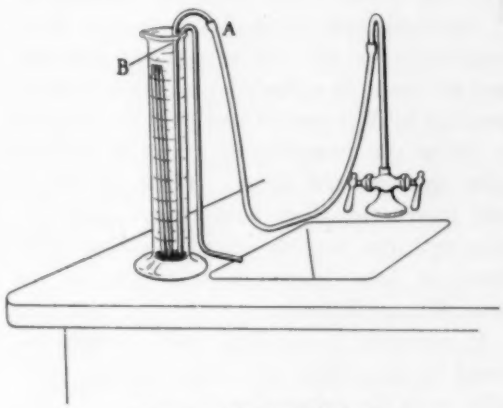

Fig. 1.—Pipette Washer

A good technique in an average size laboratory is to place the wire or glass gauze in the bottom of the cylinder and fill it half-full of disinfectant solution. This will prevent blood, milk, and other solutions from drying on the pipettes and making them hard to clean. At the close of the day the cylinder is carried to the sink and the pipette washer inserted and turned on. The next morning the pipettes will have rinsed about one hundred to two hundred times. To wash milk pipettes or other hard to clean or greasy ones, Calgonite with a wetting agent, or other good cleaning powders, should be used instead of lysol or other disinfectant solutions.

REFERENCE

(1) Wadsworth, August B., "Standard Methods of the Division of Laboratories and Research of the New York State Department of Health," ed. 2, The Williams & Wilkins Company, Baltimore, Md., 1939, p. 132.

* Received Aug. 15, 1946, from the Biological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

Stability of Penicillin in Solutions of Ephedrine*

By VICTOR P. SEEBERG, DORIS JANE BROWN, and FREDERICK F. JOHNSON

The stability of penicillin in solutions of ephedrine has been determined. At refrigerator temperatures the stability of penicillin is not appreciably affected by ephedrine. At room and incubator temperatures ephedrine exerts an appreciable effect on the stability of penicillin in some preparations. This effect is greatest on crystalline sodium penicillin and commercial calcium penicillin and least when commercial sodium penicillin is used.

IN TREATING conditions of sphenoethmoiditis with penicillin, relief of nasal congestion by the use of a local vasoconstrictor such as ephedrine has been recommended by Woodward and Holt (1) in order to allow the penicillin to come in contact with the infected areas. These investigators instructed their patients to spray the nose with the vasoconstrictor solution every three or four hours in addition to the penicillin application.

It occurred to us that such medication could be simplified by combining the penicillin with the ephedrine solution, providing the stability of the penicillin is not adversely affected during the period of use.

Since both the sodium and calcium salts of penicillin are available commercially, it seemed desirable to obtain stability information or combinations containing each of these salts. In addition, the stability of crystalline penicillin G[1] was also studied under these conditions. The sodium penicillin and calcium penicillin were representative of commercial production lots, the former assaying 933 units per milligram and the latter 754 units per milligram. The crystalline penicillin G assayed 1550 units per milligram.

EXPERIMENTAL

A solution containing one per cent ephedrine sulfate in isotonic solution of sodium chloride was prepared and divided into three portions. To each portion was added 1000 units per cc. of sodium penicillin, calcium penicillin, or crystalline sodium penicillin G and adjusted to *p*H 6.3, then sterilized by Seitz filtration. A control solution of each penicillin salt was also prepared in isotonic solution of sodium chloride and treated in the same manner. Stability samples of each of the six preparations were stored at 37°, 23°, and 5°. Samples were removed for assay at the beginning of the storage period and at intervals thereafter. The 37° samples were tested daily, the 23° samples were tested every three days and the 5° samples were tested every seven days. The 37° samples were followed for ten days, the 23° samples for thirty days and the 5° samples for fifty-six days. All samples were tested in quadruplicate and an average of the four assays was used in plotting the stability curves. The assay method used was the standard F. D. A. cylinder-plate method. Results are shown in Figs. 1, 2, and 3.

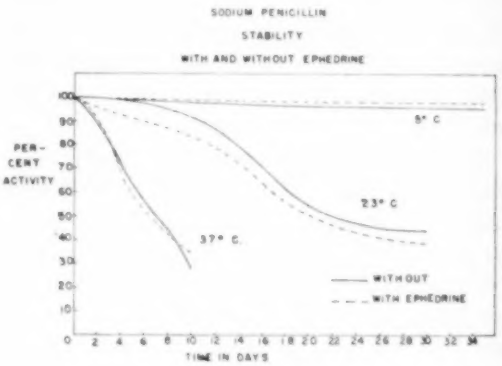

FIGURE 1

DISCUSSION

There is no appreciable effect of ephedrine sulfate on penicillin stability at refrigerator temperature in any of the preparations. In certain preparations, some effect on stability is evident at room temperature and incubator temperature. This effect is more evident in the cases of the crystalline sodium penicillin G and commercial calcium penicillin, and least evident in the case of the commercial sodium penicillin. Since the biological assay of penicillin under ordinary laboratory conditions is usually subject to at least 5 per cent error, and occasionally to as much as 15 per cent error, an approximation of the time necessary for actual

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[1] Prepared by H. N. Beniams of these laboratories.

potency loss in these preparations might be obtained by noting the time necessary to lose over 15 per cent of the original potency as determined by our method of assay. Such an analysis is made in Table I.

It is of interest to note the stability of the penicillin control solutions at the various temperatures of storage because of the frequency with which this question arises in the laboratory and the pharmacy. After five weeks' storage at 5°, the solution of commercial sodium penicillin assayed 96 per cent of its original potency, falling to 93 per cent after eight weeks. After four weeks' storage at 5° the solution of commercial calcium penicillin assayed 103 per cent of its original potency, falling to 93 per cent after five weeks. Therefore, taking into consideration the probable error of assay, it seems safe to say that these solutions retained their full potency for approximately one month. The solution of crystalline sodium penicillin G assayed 93 per cent of its original potency at the end of one week, 89 per cent at the end of three weeks, and 83 per cent at the end of five weeks. Therefore, its stability in solution appears to be slightly inferior to the commercial salts, the period of full potency under refrigeration being in the neighborhood of one or two weeks. The stabilities of the control solutions at 23° are considerably less than at 5°, all losing 15 per cent of their original potency in seven to twelve days. At 37°, the average time to lose 15 per cent of the original potency was about two days in the case of the commercial sodium and calcium salts and about one day in the case of the crystalline sodium penicillin G.

TABLE I.—COMPARATIVE RATES OF POTENCY LOSS, VARIOUS PENICILLIN SALTS WITH AND WITHOUT EPHEDRINE

Preparation	Days to Lose 15 Per Cent Potency		
	37° C.	23° C.	5° C.
Sodium penicillin	2	12	Over 56
Sodium penicillin with ephedrine	2	8	Over 56
Calcium penicillin	2	7	35
Calcium penicillin with ephedrine	1	3	30
Crystalline penicillin G	Less than 1	7	28
Crystalline penicillin G with ephedrine	Less than 1	3	27

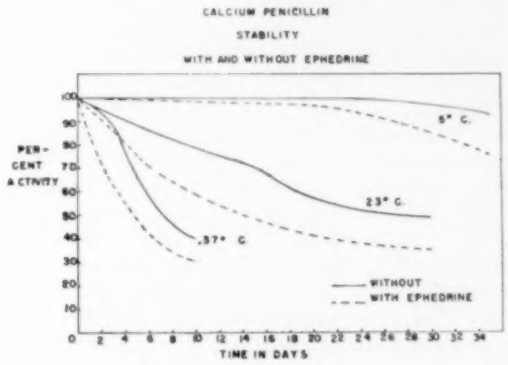

FIGURE II

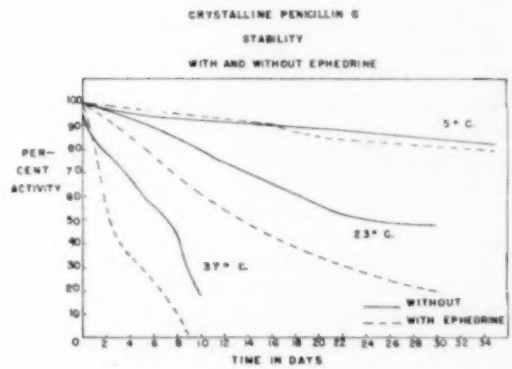

FIGURE III

CONCLUSIONS

1. The addition of 1 per cent ephedrine sulfate to solutions of commercial sodium penicillin, commercial calcium penicillin, or crystalline sodium penicillin G has no appreciable effect on the stability of the penicillin at 5° for ordinary periods of storage.

2. When stored at higher temperatures (23° or 37°) ephedrine sulfate lowers the stability of penicillin solutions to some extent, the effect being greater on solutions of commercial calcium penicillin and crystalline sodium penicillin G than on commercial sodium penicillin.

3. Crystalline sodium penicillin G in solution appears to be slightly less stable than commercial sodium or calcium penicillins.

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The Synthesis of Chemotherapeutic Agents. I.* The Synthesis of Certain Thio and Dithio Compounds†,‡

By JOHN E. CHRISTIAN, GLENN L. JENKINS, LeROY C. KEAGLE, and JEAN ANN CRUM

Investigation of 5,5′-diacetamido-8,8′-diquinolyl disulfide indicated that it possesses a relatively high antimalarial activity. The report of this activity stimulated interest in the possible chemotherapeutic value of certain related thio and dithio compounds. This paper, the first of a series, describes the synthesis of sixteen compounds, eight of which are new, related to 5,5′-diacetamido-8,8′-diquinolyl disulfide. Twelve of these compounds were tested for antimalarial activity and the results of these tests are tabulated. In addition some of the compounds were also subjected to screening tests against Trypanosoma brucei, influenza virus and tetanus toxin.

A REPORT on the relatively high antimalarial activity of 5,5′-diacetamido-8,8′-diquinolyl disulfide (1) aroused considerable interest in the synthesis and chemotherapeutic activity of certain related thio and dithio compounds. This paper is the first of a series describing the synthesis and chemotherapeutic activity of a number of these compounds.

The compounds reported here have been prepared in an effort to obtain substances of higher intrinsic activity and with better pharmacological properties. In addition to the usual antimalarial tests, some of these compounds have been subjected to screening tests against Trypanosoma brucei, influenza virus, and tetanus toxin.

Most of the compounds have been synthesized by methods described by Christian and Jenkins (3, 4). Compounds containing active halogens were treated with sodium disulfide, splitting out sodium chloride to form thio or dithio compounds. If there were nitro groups present, these were reduced to the corresponding amino compounds using stannous chloride and hydrochloric acid or sodium sulfide.

EXPERIMENTAL

1. 5,5′-Diamino-8,8′-diquinolyl Disulfide.—Two reduction procedures were found to give more satisfactory yields than previously reported. (A) A modification of the procedure using stannous chloride and hydrochloric acid described by Christian and Jenkins (2) and (B) the use of sodium sulfide (3).

Procedure A.—Three-tenths gram of 5,5′-dinitro-8,8′-diquinolyl disulfide, the preparation of which was reported in a previous reference (4), was added with stirring all at once to a solution of 11.5 Gm. of $SnCl_2.2H_2O$ in 12 cc. of concentrated HCl cooled to 5°. The mixture was heated on a steam bath for one hour, cooled, and placed in the cold overnight. The precipitate was collected, suspended in 20 cc. of water, and 27 cc. of 50% NaOH added with cooling. Water (140 cc.) was added to insure complete solution of the sodium stannate formed. The solution was filtered and air bubbled slowly through the filtrate for twenty-four hours (hydrogen peroxide may be used instead of air for the oxidation of the thiol to the disulfide). The yellow disulfide which precipitates was filtered and recrystallized from acetone and water. The pure crystals melt at 225–226° with decomposition.

2. 5,5′-Diacetamido-8,8′-diquinolyl Disulfide.—The procedure used was that described by Christian and Jenkins (2).

3. 5,5′ - Bis(*p*-acetamido-benzenesulfonamido)-8,8′-diquinolyl Disulfide.—The procedure used was that described by Christian and Jenkins (2).

4. 6,6′ - Dinitro - 2,2′ - diquinolyl Sulfide.—The starting material, 2-chloro-6-nitroquinoline, for this reaction was supplied by the Committee on Medical Research (CMR) of the Office of Scientific Research and Development (OSRD). On recrystallization from alcohol and acetone this substance melted at 235° (total immersion).

To 1000 cc. of ethyl alcohol was added 20 Gm. of 2-chloro-6-nitroquinoline and the mixture heated to reflux. Through a dropping funnel placed above the condenser a hot alcoholic solution of Na_2S_2, prepared by dissolving 11.2 Gm. of crystalline sodium sulfide ($Na_2S.9H_2O$) and 1.5 Gm. of sulfur in 115 cc. of hot alcohol, was added over a period of

* For a previous paper in this series, see Christian and Jenkins, THIS JOURNAL, **34**, 147–49(1945).

† Received July 15, 1946, from Purdue University, School of Pharmacy, Lafayette, Ind.

Presented to the Scientific Section of the A. PH. A., Pittsburgh meeting, 1946.

‡ The work described in this paper was done in collaboration with Eli Lilly and Company and the Committee on Medical Research of the Office of Scientific Research and Development.

one hour to the boiling mixture. After the addition was complete, the alcoholic mixture was refluxed for four hours. The mixture was cooled and the product separated by filtration, washed with water, and dried; 18.5 Gm. yield (48% of the theoretical). Several recrystallizations from nitrobenzene or a mixture of nitrobenzene and pyridine yielded lemon-yellow crystals melting at 254–255° (total immersion).

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S$: N, 14.82%. Found: N, 14.22%.

5. 6,6′-Diamino-2,2′-diquinolyl Sulfide.—To a solution of 46 Gm. of $SnCl_2.2H_2O$ in 48 cc. of concentrated HCl cooled to 5° was added all at once with stirring 4.3 Gm. of 6,6′-dinitro-2,2′-diquinolyl sulfide. The mixture was heated on a steam bath for two hours, cooled, and filtered. The tin salt collected was suspended in 80 cc. of water, and 110 cc. of 50% NaOH added with cooling. Water (500 cc.) was added and the mixture filtered. The yellow precipitate was recrystallized from acetone and water using decolorizing charcoal to aid purification. Yield 5.5 Gm. (53.7% of the theoretical), m. p. 241–242° (total immersion).

Anal.—Calcd. for $C_{18}H_{14}N_4S$: S, 10.06%. Found: S, 10.10%.

6. 8,8′ - Dinitro - 7,7′ - diquinolyl Sulfide.—The starting material, 7-chloro-8-nitroquinoline, was supplied by CMR; m. p. 182–184°.

To 200 cc. of alcohol was added 20 Gm. of 7-chloro-8-nitroquinoline and the mixture heated to reflux. A hot alcoholic solution of Na_2S_2, prepared by dissolving 11.1 Gm. of $Na_2S.9H_2O$ and 1.5 Gm. sulfur in 110 cc. of hot alcohol, was added over a period of one hour. After the addition was complete the mixture was refluxed for four hours. The mixture was cooled and the product separated by filtration, washed with water and dried; 14.5 Gm. yield (38% of the theoretical). Several recrystallizations from pyridine and alcohol yielded a product melting at 265–266° (total immersion).

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S$: S, 8.47%; N, 14.82%. Found: S, 8.63%; N, 14.09%.

7. 8,8′ - Diamino - 7,7′ - diquinolyl Sulfide.—Pure 8,8′-dinitro-7,7′-diquinolyl sulfide (13.3 Gm.) was added slowly with cooling to a solution of 72 Gm. $SnCl_2.2H_2O$ in 70 cc. of concentrated HCl. The mixture was heated on a steam bath for two hours, cooled, filtered, and dried. Twenty-nine grams of the tin complex was obtained. This complex was heated with 250 cc. of 2.0% NaOH and the mixture extracted with acetone. The product was precipitated from the acetone upon the addition of water; 9.4 Gm. crude sulfide. Several recrystallizations from acetone and water mixtures yielded 6.5 Gm. (56.7% of the theoretical) of pure sulfide melting at 149–50° (total immersion).

Anal.—Calcd. for $C_{18}H_{14}N_4S$: S, 10.06%; N, 17.61%. Found: S, 9.80%; N, 17.49%.

8. β,β′ - Dianthraquinone Disulfide.—β-Chloroanthraquinone (30 Gm.) was dissolved in 150 cc. of refluxing alcohol and to this hot solution was added a warm solution of Na_2S_2, prepared by dissolving 14.6 Gm. $Na_2S.9H_2O$ and 1.9 Gm. sulfur in 140 cc. of alcohol. The mixture was refluxed for four hours, cooled, and filtered. The precipitate was washed with water and alcohol and dried. The product, 2.3 Gm. (39% of theory), was recrystallized from a pyridine and alcohol mixture, m. p. 264–265° (total immersion). Gatterman (10) reported a melting point of 257°.

Anal.—Calcd. for $C_{28}H_{14}O_4S_2$: S, 13.39%. Found: S, 13.34%.

9. 2,2′-Dichloro-dibenzyl Disulfide.—This compound was prepared by Speroni (5) by a different procedure. *o*-Chlorobenzyl-chloride (30 Gm.) was dissolved in 150 cc. of refluxing alcohol. To the hot alcoholic solution a warm solution of Na_2S_2, prepared by dissolving 21.8 Gm. of $Na_2S.9H_2O$ and 2.9 Gm. sulfur in 210 cc. of hot alcohol, was added slowly. The refluxing was continued for one hour and the mixture allowed to cool. The precipitate was washed with water and recrystallized from alcohol; 28 Gm. (96% of theory), m. p. 89–90° (total immersion).

10. 4,4′ - Dichloro-dibenzyl Disulfide.—This compound was prepared by Jackson and White (6). *p*-Chlorobenzylchloride (30 Gm.) was dissolved in 150 cc. of refluxing alcohol, and the procedure carried out as described above. The product, 27 Gm. (93% of theory), was recrystallized from alcohol; m. p. 58–59° (total immersion).

11. 3,3′,4,4′ - Tetrachloro-dibenzyl Disulfide.—3,4-Dichlorobenzyl chloride (30 Gm.) was refluxed with 150 cc. of alcohol for one-half hour. To the hot alcoholic solution was added a warm solution of Na_2S_2, prepared by dissolving 17.9 Gm. of $Na_2S.9H_2O$ and 2.4 Gm. sulfur in 170 cc. of hot alcohol. The refluxing was continued for one hour and the mixture allowed to cool. The precipitate which formed was washed with water and recrystallized from alcohol; 26 Gm. (89% of theory), m. p. 94–95° (total immersion).

Anal.—Calcd. for $C_{14}H_{10}Cl_4S_2$: S, 16.66%; Cl, 37.00%. Found: S, 16.81%; Cl, 37.08%.

12. 2,2′,4,4′-Tetrachloro-dibenzyl Disulfide.—2,4-Dichlorobenzyl chloride (30 Gm.) was refluxed with 150 cc. of alcohol for one-half hour, and the procedure continued as described above. The product, 25 Gm. (84% of theory), was recrystallized from alcohol; m. p. 74–75° (total immersion).

Anal.—Calcd. for $C_{14}H_{10}Cl_4S_2$: S, 16.66%; Cl, 37.00%. Found: S, 16.75%; Cl, 37.09%.

13. 8,8′ - Dinitro - 5,5′ - diisoquinolyl Sulfide.—Ethyl alcohol (200 cc.) and 20 Gm. of 5-chloro-8-nitro-isoquinoline were placed in a flask and refluxed for one-half hour. To this hot solution was added slowly a warm solution of Na_2S_2, prepared by dissolving 11.1 Gm. of $Na_2S.9H_2O$ and 1.5 Gm. of sulfur in 110 cc. of hot alcohol. The mixture was refluxed for four hours, cooled, and filtered. The yellow product, 15.8 Gm. (40.2% of theory), was recrystallized from pyridine and alcohol; m. p.

242–244° (total immersion). Attempts to reduce this compound to the corresponding amino compound failed.

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S$: S, 8.47%; N, 14.82%. Found: S, 8.80%; N, 14.97%.

14. 5,5′-Dinitro-1,1′-diisoquinolyl Disulfide.—1-Chloro-5-nitroisoquinoline (20 Gm.) was dissolved in 500 cc. of hot alcohol. To this hot solution was added a warm solution of Na_2S_2, prepared by dissolving 11.1 Gm. $Na_2S.9H_2O$ and 1.5 Gm. sulfur in 110 cc. of hot alcohol. The mixture was refluxed for four hours, cooled, and filtered. The product, 16.3 Gm. (41.2% of theory), was recrystallized from a mixture of pyridine and alcohol, m. p. 265–266° (total immersion).

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S_2$: S, 15.61%; N, 13.66%. Found: S, 15.44%; N, 13.84%.

15. 2,2′,4,4′-Tetranitrodiphenyl Disulfide.—A modification of the procedure described by Elgersma (7) and Teppema and Sebrell (8) was used. The starting materials were 2,4-dinitrochlorobenzene (53.4 Gm.), alcohol (675 cc.), crystalline $Na_2S.9H_2O$ (32 Gm.), and sulfur (4 Gm.). The product, after washing with water, alcohol, and ether, was almost pure and weighed 43.5 Gm. (83% of theory). After recrystallization from nitrobenzene the product decomposed at 280°. Elgersma (7) reports the decomposition point as 280°.

16. 2,2′,4,4′-Tetra-aminodiphenyl Disulfide.—2,2′,4,4′-Tetranitrodiphenyl disulfide (12 Gm.) was mixed with a solution of 91 Gm. $SnCl_2.2H_2O$ in 95 cc. of concentrated HCl. The mixture was heated on a steam bath for twenty hours, cooled, filtered, and the filtrate diluted with water and made alkaline with 15% NH_4OH. Air was passed through the solution for twenty-four hours and the product (1.5 Gm.) filtered off. Recrystallization from a mixture of benzene and alcohol gave a product melting at 147–148° (total immersion). Muller (9) reported a melting point of 146–147°.

Chemotherapeutic Activity.[1]—Table I indicates the antimalarial activity of some of the compounds prepared.

Compounds numbered CJ-2, CJ-1, CJ-115-2, CJ-601-1, and CJ-3 (Table I) have been tested against tetanus toxin in mice. None of the drugs were effective.

Compounds numbered CJ-2, CJ-1, CJ-106-2, CJ-115-2, CJ-601-1, and SN 8167 have been subjected to standardized Swiss mouse therapeutic tests against influenza virus. None of the drugs have produced any significant cures.

Therapy tests with compound number SN 8167 were conducted on rats infected with Trypanosoma brucei, with intraperitoneal doses ranging from 50 mg. to 75 mg. per rat. No trypanocidal action was detected.

[1] We are indebted to Eli Lilly and Company for permission to quote the results of chemotherapeutic tests and for much helpful discussion.

TABLE I

Name	Number	Antimalarial Activity[b]
5,5′-Diamino-8,8′-diquinolyl disulfide	SN 8167[a]	Q, 1.07 Toxicity LD_{50} 1760 mg./Kg.
5,5′-Diacetamido-8,8′-diquinolyl disulfide	SN 9583	Q, 4.0 Toxicity LD 2500 mg./Kg.
5-(*p*-Aminobenzenesulfonamido)-8-chloroquinoline[c]	CJ-1	Inactive 1–50 Gm. Q, 0.16 i Toxicity LD_{50} 2500 mg./Kg.
5-(*p*-Acetamidobenzenesulfonamido)-8-chloroquinoline[c]	CJ-2	Inactive 5–200 mg. Q, 0.08 i
5,5′-*bis*(*p*-Acetamidobenzenesulfonamido)-8,8′-diquinolyl disulfide	CJ-5	Inactive 1–50 mg. Q, 0.16 i
8,8′-Diamino-7,7′-diquinolyl sulfide	CJ-105–2	Q, 0.2 i
Sodium-5-amino-quinoline-8-sulfonate[c]	CJ-116–1	Q, 0.2 i
2,2′,4,4′-Tetrachlorodibenzyl disulfide	CJ-243–1	Inactive 5–100 mg. Q, 0.12 i
3,3′,4,4′-Tetrachlorodibenzyl disulfide	CJ-244–1	Inactive 5–100 mg. Q, 1.12 i
2,2′-Dichloro-dibenzyl disulfide	CJ-245–1	Inactive 5–100 mg. Q, 0.12 i
4,4′-Dichloro-dibenzyl disulfide	CJ-246–1	Inactive 5–100 mg. Q, 0.12 i
β,β′-Dianthraquinone disulfide	CJ-601–1	Inactive 5–100 mg. Q, 0.16 i

[a] SN = Survey number, an identifying number for compounds which will appear in a forthcoming monograph entitled "A Survey of Antimalarial Drugs, 1941–1945," F. Y. Wiselogle, Editor.

[b] Antimalarial activity as reported by Eli Lilly and Company. The screening tests were obtained with ducks infected with Plasmodium lophurae.

[c] Compounds, the synthesis of which have been previously reported by Christian and Jenkins (2).

SUMMARY

1. The synthesis of a number of new thio and dithio compounds has been described.

2. The antimalarial activity and results of other chemotherapeutic tests of these compounds have been presented.

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The Synthesis of Chemotherapeutic Agents. II.* The Synthesis of Certain Thio and Dithio Compounds†,‡

By JOHN W. WETZEL, DONALD E. WELDTON, JOHN E. CHRISTIAN, GLENN L. JENKINS, and G. BRYANT BACHMAN

In this paper, the second of a series, the continuation of a study of certain thio and dithio compounds related to 5,5′-diacetamido-8,8′-diquinolyl disulfide is described. Of the fourteen sulfur compounds synthesized and characterized, which are described in this paper, twelve are new. Several of these have undergone screening tests for antimalarial activity. Incidental to the syntheses reported new methods are described for the preparation of 2,3- and 4-chloro isomers of 5-nitroquinoline.

THIS IS a report on the continuation of a study of certain thio and dithio compounds the preparation of which were indicated in an effort to produce compounds of higher pharmacological activity and better pharmacological properties than a related compound, 5,5′-diacetamido-8,8′-diquinolyl disulfide, which has been shown to possess antimalarial activity (1).

In this paper 14 additional compounds have been described, some of which have been tested for antimalarial activity.

EXPERIMENTAL

1. 3,3′-Dinitro-4,4′-diquinolyl Sulfide.—A solution of 12.0 Gm. of $Na_2S.9H_2O$ in 30 cc. of water was diluted with 100 cc. of methanol; 1.60 Gm. of powdered sulfur was added, and the mixture warmed until solution was complete. The resulting solution of Na_2S_2 was cooled to room temperature and added slowly to a well-stirred and cooled solution of 20.8 Gm. of powdered 3-nitro-4-chloroquinoline in 40 cc. of chloroform, keeping the temperature at 20–25°. The mixture was stirred for thirty minutes after all the Na_2S_2 was added and the precipitate of 3,3′-dinitro-4,4′-diquinolyl sulfide filtered off, washed with methanol, stirred with excess dilute NaOH, washed with water, and dried. Yield 19.7 Gm. (96% of theory) of yellow crystals which began to decompose and darken at about 200° and eventually melted with decomposition. No definite melting point could be obtained even after several recrystallizations from toluene.

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S$: N, 14.81%. Found: N, 14.84%.

Comments: It was expected that the above reaction would yield the disulfide. Analysis indicated that the product was the sulfide. This was confirmed by substituting Na_2S for Na_2S_2 in the synthesis.

A mixture of 20.8 Gm. of 3-nitro-4-chloroquinoline in 40 cc. of chloroform was stirred while a solution of 12.0 Gm. of $Na_2S.9H_2O$ in 30 cc. of H_2O and 100 cc. of methanol was added dropwise for ten to fifteen minutes, keeping the temperature below 25°. The mixture was stirred for thirty minutes after all the Na_2S had been added, then filtered. The residue was washed with methanol, suspended in water, and made alkaline with NaOH, filtered, and the residue washed with water and dried. The yield of 3,3′-dinitro-4,4′-diquinolyl sulfide was 17.0–18.0 Gm. (90–95% of theory). The product was identical with the product from the Na_2S_2 reaction.

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S$: N, 14.81%. Found: N, 14.77%.

2. 3-Amino-4-quinolinethiol.—A mixture of 18.9 Gm. of crude 3,3′-dinitro-4,4′-diquinolyl sulfide, 92 Gm. (10% excess) of $Na_2S.9H_2O$, and 300 cc. of 50% alcohol was stirred and heated slowly to boiling, refluxed for two hours, cooled slightly, slowly acidified with 100 cc. of concentrated HCl, and cooled to room temperature. The 3-amino-4-quinolinethiol hydrochloride was filtered off, washed with 50% alcohol, and suspended in 200 cc. of water. Concentrated NaOH was added until the solution was strongly basic and 1 to 2 Gm. of anhydrous Na_2SO_3 was added and the mixture stirred until the orange thiol had disappeared. The sulfur was filtered off, the filtrate treated with charcoal without heating and filtered again. The clear filtrate was treated, with stirring, with glacial acetic acid, added dropwise until the *p*H fell to about 5. The mixture was allowed to stand for fifteen to thirty minutes and filtered. The crystalline product was washed with water and dried over P_2O_5. Yield 16.2–17.6 Gm. (92–100% of the theory) of orange needles, which began to decompose at about 190° and slowly carbonized. On recrystallization the product was easily oxidized to the disulfide; therefore the crude product was used for further synthesis.

3. 3,3′-Diamino-4,4′-diquinolyl Disulfide.—A solution of 16.6 Gm. of crude 3-amino-4-quinoline-

* For previous papers in this series see Christian and Jenkins, THIS JOURNAL, **34**, 147–49(1945), and **35**, 328(1946).

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‡ The work described in this paper was done in collaboration with Eli Lilly and Company and the Committee on Medical Research of the Office of Scientific Research and Development.

thiol in 60 cc. of pyridine was stirred and cooled while 12.0 Gm. of solid I_2 was added slowly. The solution became deep red and heat was evolved. Ten grams of Na_2CO_3 dissolved in 100 cc. of water was added slowly, the mixture stirred, allowed to cool to room temperature, filtered and the residue washed well with water. The yellow crystals, after drying at 60°, became orange in color and melted at 123–125°, resolidified to an orange solid and melted at 157°. Heated slowly, the crystals turned orange above 100° and melted at 157°; yield 16.0 Gm. (96% of theory). If recrystallized from alcohol and water the m. p. is raised to 169–171° (decomp.).

Anal.—Calcd. for $C_{18}H_{14}N_4S_2$: N, 16.00%. Found: N, 15.89%.

4. 3-Acetylamino-4-quinolinethiol.—A solution of 16.7 Gm. of crude 3-amino-4-quinolinethiol in 40 cc. of pyridine was stirred vigorously while 20 cc. (100% excess) of acetic anhydride was added rapidly. The mixture was stirred ten to fifteen minutes, 150 cc. of boiling water added, stirred, and cooled to room temperature. The product was filtered off, washed with water and dried. Yield, 19.9 Gm. (96% of theory). Recrystallization from pyridine yielded pure 3-acetylamino-4-quinolinethiol as yellow needles which darkened about 250° and melted with evolution of gas at about 260–264°.

Anal.—Calcd. $C_{11}H_{10}N_2OS$: N, 12.84. Found: N, 12.83.

5. 5-Nitroquinoline.—The procedure used for the nitration of quinoline was a combination of that used by Dufton (4) and Fieser and Hershberg (5). The modifications were made in an effort to obtain as large a yield of 5-nitroquinoline as possible.

One hundred grams (0.775 mole) of synthetic quinoline was placed in an evaporating dish cooled with cold water. Sixty-six grams (20% excess) of fuming nitric acid was added dropwise with continuous agitation. The quinoline nitrate was broken up into small pea-sized particles and divided into ten equal portions. These portions were added to 100 cc. of concentrated H_2SO_4 which was contained in a suitable flask cooled in an ice bath. After each portion of the nitrate was added, 10 cc. of 20% oleum was stirred in controlling the temperature of the mixture between 10° and 20°. After all the reactants were in the flask the mixture was stirred for one hour, keeping the temperature at 10–20°. The liquid was poured into about 800 Gm. of crushed ice and the solution neutralized with 500–600 cc. of NH_4OH. The solid which formed on cooling was separated and dissolved in 670 cc. of hot dilute HNO_3 (d. 1.12). The solution was cooled and a yellow solid crystallized. This solid was dissolved in hot water and neutralized with NH_4OH. An oil separated and crystallized on cooling. The yield of pure 5-nitroquinoline was 50 Gm. (36.5% of theory). The nitrate melted at 186–186.5°, and the free base at 71–72°.

6. Perphthalic Acid.—Perphthalic acid was prepared according to the procedure described by Cooper (6), treating phthalic anhydride with H_2O_2 in NaOH solution. The peracid content determined by iodometric titration corresponded to a 70% yield.

7. 5-Nitroquinoline N-Oxide Phthalate.—Sixty grams (0.35 mole) of 5-nitroquinoline was dissolved in 700 cc. of ether which contained 95 Gm. (0.52 mole) of perphthalic acid and the mixture was allowed to stand for twenty-four hours at 15°. A yellow precipitate gradually formed, giving a quantitative yield of crude product.

8. 5-Nitroquinoline N-Oxide.—A solution of one liter of water and 85 Gm. of sodium carbonate was stirred vigorously as 152.6 Gm. of powdered 5-nitroquinoline N-oxide phthalate was added slowly. The mixture was stirred for forty-five minutes and then the orange-yellow solid was filtered out and washed with ice water until the washings were neutral and colorless. The yield was 67% of crude product. Recrystallization from toluene gave a product melting at 163–164°.

Anal.—Calcd. for $C_9H_6N_2O_3$: C, 56.85; H, 3.18. Found: C, 56.86; H, 3.26.

9. 2-Chloro-5-nitroquinoline, 3-Chloro-5-nitroquinoline, and 4-Chloro-5-nitroquinoline.—Fifty grams (0.26 mole) of finely powdered 5-nitroquinoline N-oxide was dissolved slowly in 225 cc. of phosphorus oxychloride contained in a cooled flask under reflux. The flask was transferred to an oil bath heated to 60° and the temperature slowly raised to 105–115° for two hours. The excess $POCl_3$ was removed by vacuum distillation and the residue poured onto 500 Gm. of crushed ice. The mixture was stirred for two hours at 5–10° to hydrolyze the $POCl_3$. The insoluble material, 2-chloro-5-nitroquinoline, was filtered off and washed with cold water until the washings were neutral. The washings were added to the mother liquor and the total volume made up to one liter. A small amount of a solid mixture of 2- and 3-chloro-5-nitroquinolines separated and was filtered off, and the filtrate diluted to two liters total volume. More solid, a mixture of 3- and 4-chloro-5-nitroquinolines, separated and was filtered off. The filtrate was kept cold while 100 cc. of concentrated ammonium hydroxide was added dropwise. A mixture of 3- and 4-chloro-5-nitroquinolines separated. The filtrate was allowed to stand in the cold and filtered to remove a mixture of 4-chloro-5-nitroquinoline and an alkali soluble impurity.

The fractions were treated chemically to separate the isomers. 2-Chloro-5-nitroquinoline was separated from 3-chloro-5-nitroquinoline by virtue of the insolubility of the 2-isomer in 6 *N* HCl. The mixture was dissolved in concentrated HCl and then diluted with an equal volume of water. The 2-isomer separated from this solution.

3-Chloro- and 4-chloro-5-nitroquinolines were separated through the insolubility of the 3-isomer in 3 *N* HCl. The mixture was stirred well with 3 *N* HCl, and the insoluble 3-isomer filtered off. The filtrate was slowly neutralized in the cold with NH_4OH to precipitate 4-chloro-5-nitroquinoline.

The identity of the compounds was established as follows:

2-Chloro-5-nitroquinoline obtained in 44.5% yield was recrystallized from methanol and melted at 133–134°, literature value 129° (2, 3). A mixed melting point with material prepared at the University of Virginia (O. S. R. D.) showed no depression. Hydrolysis of the compound with concentrated HCl gave 2-hydroxy-5-nitroquinoline, m. p. 301–303°, literature value 298–299° (2, 3).

3-Chloro-5-nitroquinoline, obtained in 13.1% yield was recrystallized from ethanol and melted at 128–129°, literature value 127–128° (7). This compound could not be hydrolyzed on prolonged treating with concentrated HCl.

4-Chloro-5-nitroquinoline obtained in 9.4% yield was recrystallized from methanol, m. p. 149–150°.

Anal.—Calcd. for $C_9H_5N_2O_2Cl$: C, 51.82; H, 2.42. Found: C, 51.76; H, 2.45.

The chloro compound was hydrolyzed by heating with concentrated HCl to give 4-hydroxy-5-nitroquinoline which had an indefinite decomposition point of 335–340°.

Anal.—Calcd. for $C_9H_6N_2O_3$: C, 56.84; H, 3.18. Found: C, 56.86; H, 3.40.

10. 5,5′-Dinitro-2,2′-diquinolyl Sulfide.—The starting material, 2-chloro-5-nitroquinoline, used in this synthesis was obtained from two sources. A 45-Gm. sample was obtained from O. S. R. D. It was purified by recrystallization from alcohol until the product melted at 127–129°. The melting point for the pure material has been reported as 130° (2, 3). A second portion was prepared by the chlorination of the N-oxide of 5-nitroquinoline.

A solution of 5.2 Gm. of 2-chloro-5-nitroquinoline in 75 cc. of alcohol was refluxed and stirred. A solution of sodium disulfide (prepared from 3 Gm. of $Na_2S.9H_2O$ and 0.4 Gm. of sulfur in 25 cc. of alcohol and 6 cc. of water) was added slowly during forty-five minutes. The solution was allowed to reflux for eleven hours and cooled in an ice chest overnight. The yellow precipitate was filtered off, rinsed with alcohol, triturated with dilute NaOH, washed with water and alcohol and dried. Two and two-tenths grams of sulfide was obtained which on recrystallization from pyridine melted with decomposition at 260–261°. The NaOH filtrate was acidified with glacial acetic acid yielding an orange precipitate of 5-nitro-2-quinolinethiol; m. p. 234–236°. Additional quantities of these products were obtained by working up the original filtrate. The combined yield of crude 5,5′-dinitro-2,2′-diquinolyl sulfide was 3.7 Gm. (73% of theory). The total 5-nitro-2-quinolinethiol obtained was 0.9 Gm. (21% of theory).

Anal.—Calcd. for $C_{18}H_{10}N_4O_4S$: C, 57.14; H, 2.67. Found: C, 57.12; H, 2.77.

11. 5 - Acetamido - 2 - chloroquinoline.—Eight grams of 2-chloro-5-nitroquinoline was dissolved in 140 cc. of glacial acetic acid and 20 cc. of acetic anhydride (50% excess). Approximately 1 Gm. of Raney nickel catalyst was added and the mixture reduced at low pressure and room temperature. The reaction mixture was filtered, treated with Norite, and filtered again. The filtrate was slowly neutralized with NH_4OH as it was stirred in an ice bath. A white solid gradually precipitated as the solution approached neutrality. The solid was filtered and washed with water. The material was recrystallized from ethanol to give a product, m. p. 214–215°, yield 75% of theory.

Anal.—Calcd. for $C_{11}H_9N_2OCl$: C, 59.93%; H, 4.11%. Found: C, 59.92%; H, 4.38%.

12. 5-Acetamido-2-quinolinethiol.—Two grams 5-acetamido-2-chloroquinoline was dissolved in 35 cc. of ethanol by heating to reflux. To this hot solution an alcoholic solution of Na_2S_2 was added dropwise over a period of five hours. The Na_2S_2 solution was prepared by dissolving 1.07 Gm. of $Na_2S.9H_2O$ in 10.2 cc. of ethanol and 3.4 cc. of water and then adding 0.14 Gm. of sulfur. The mixture was refluxed with stirring for twenty hours. The yellow solid was filtered off and dissolved in 10% NaOH. The alkaline solution was treated with Norite and the yellow solid reprecipitated with acetic acid; yield 51.5% of theory. Recrystallization from alcohol yielded a product melting at 274–278° with decomposition.

Anal.—Calcd. for $C_{11}H_{10}N_2OS$: S, 14.69%. Found: S, 14.72%.

13. Bis(6 - methoxy - 2 - methyl - 7,8,9,10 - tetrahydro-4-benzo(h)quinolyl) Sulfide.—To 100 cc. of warm alcohol, 5.23 Gm. of 4-chloro-6-methoxy-2-methyl-7,8,9,10-tetrahydrobenzo(h)quinoline was added. Complete solution occurred at 60° after which a warm alcoholic solution of Na_2S_2 (prepared by dissolving 4.72 Gm. $Na_2S.9H_2O$ and 0.626 Gm. powdered S in 40 cc. of 95% alcohol) was added with stirring. The solution was refluxed seventeen hours, cooled, diluted with water to dissolve the NaCl, and filtered; yield 4.8 Gm. (89% of theory). Recrystallization from dioxan gave a product melting at 200–209°; from toluene the melting point was 211–218°.

Anal.—Calcd. for $C_{30}H_{32}O_2N_2S$: S, 6.62%. Found: S, 7.01%.

14. 3-Amino-4-quinolinesulfonic Acid.—A solution of 4.4 Gm. of 3-amino-4-quinolinethiol in 45 cc. of 50% methanol containing 1.1 Gm. (10% excess) of NaOH was cooled and stirred while 9.4 Gm. (10% excess) of 30% H_2O_2 was added dropwise keeping the temperature at 30–35°. Stirring and cooling were continued until the precipitate which formed had redissolved, and the solution then refluxed from ten to fifteen minutes. It was then acidified with 20 cc. of 50% H_2SO_4 and allowed to cool to room temperature. The yellow precipitate was filtered off, washed with methanol and dried. The product was purified by reprecipitation. The yield of pure light yellow 3-amino-4-quinolinesulfonic acid was 4.0 Gm. (72% of theory). The product has no definite melting point but darkens at about 240°, and melts with decomposition between 250–265°.

Anal.—Calcd. for $C_9H_8N_2SO_3$: S, 14.28%. Found: S, 14.33%.

SUMMARY

1. Twelve new compounds have been synthesized in an effort to find compounds with desirable chemotherapeutic properties. A new method is described for the preparation of the 2, 3 and 4-chloro-isomers of 5-nitroquinoline.

2. Sufficient quantities of these compounds have been prepared for pharmacological testing.

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The Synthesis of Chemotherapeutic Agents.* III. The Synthesis of Certain Thio Compounds†,‡

By YUEN-FU CHENG, JOHN E. CHRISTIAN, and GLENN L. JENKINS

Five new sulfa compounds possessing possible medicinal value have been synthesized and characterized.

SINCE 5,5′-diamino-8,8′-diquinolyl disulfide and some of its derivatives have been synthesized (1) and investigation has revealed possible chemotherapeutic value for these compounds (2), it is believed that 5,5′-diamino-8,8′-diquinolyl sulfide and its derivatives may have some therapeutic applications. The objective of the project reported here is the synthesis of 5,5′-diamino-8,8′-diquinolyl sulfide and some of its derivatives, so that they might be tested.

The starting material used in this synthesis was *o*-chloroaniline, from which 8-chloroquinolyl, 5-nitro-8-chloroquinoline, and 5,5′-dinitro-8,8′-diquinolyl sulfide have been made by using the procedures described previously (3–6).

The synthesis of 5,5′-diamino-8,8′-diquinolyl sulfide was accomplished when stannous chloride and hydrochloric acid were used as reducing agents to reduce 5,5′-dinitro-8,8′-diquinolyl sulfide. From 5,5′-diamino-8,8′-diquinolyl sulfide, 5,5′-diacetylamido-8,8′-diquinolyl sulfide was prepared by using acetic anhydride and anhydrous sodium acetate; 5,5′-diformylamido-8,8′-diquinolyl sulfide was synthesized by treatment with strong formic acid.

When 5,5′-diamino-8,8′-diquinolyl sulfide was condensed with two molecules of *p*-acetamidobenzene sulfonyl chloride, 5,5′-*bis*-(*p*-acetamidobenzene sulfonamido)-8,8′-diquinolyl sulfide was obtained.

EXPERIMENTAL

1. 8-Chloroquinoline.—The procedures used were those described by Urist (3), Christian (4), and Fourneau, *et al.* (5). The yield obtained was 67%.

2. 5-Nitro-8-chloroquinoline.—The procedures used were essentially those of Christian (4) and Fourneau, *et al.* (5). The yield obtained was 81% and the white needle crystals melted at 144.8°. [Urist (3) reported 145°; Fourneau (5) reported 145°.]

3. 5,5′ - Dinitro - 8,8′ - diquinolyl Sulfide.—By using the procedure described by Surrey and Lindwall (6), a 90% yield was obtained. The product melted at 288°. [Surrey and Lindwall (6) reported m. p. 288.5–290°.]

4. 5,5′-Dinitro-8,8′-diquinolyl Sulfone.—The procedure used was essentially that described by Surrey and Lindwall (6). Greenish yellow crystals were obtained; m. p. 259°. [Surrey and Lindwall (6) reported m. p. 260°.] The yield was 55.5%.

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* For previous papers in this series see THIS JOURNAL, **34**, 147(1945); **35**, 328(1946); **35**, 331(1946).

† Received July 15, 1946, from Purdue University, School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section of the A. PH. A., Pittsburgh meeting, 1946.

‡ The work described in this paper was done in collaboration with Eli Lilly and Company and the Committee on Medical Research of the Office of Scientific Research and Development.

5. 5,5′ - Diamino - 8,8′ - diquinolyl Sulfide.—A solution of 25 Gm. of stannous chloride in 50 cc. of concentrated HCl was slowly added with continuous stirring to a cooled solution of 5 Gm. of 5,5′-dinitro-8,8′-diquinolyl sulfide dissolved in 100 cc. of concentrated HCl. The yellow tin precipitate appeared gradually until the reaction was completed. The mixture was cooled in an ice bath about one hour, the precipitate was collected, dried, dissolved in about 60 cc. of 20% NaOH solution, and then cooled. The mixture was filtered and the yellow precipitate was washed with two 10-cc. portions of 20% NaOH solution, with ammonium hydroxide, and then with distilled water. The washed precipitate was dried, dissolved in acetone, and decolorized with activated charcoal. The acetone solution was concentrated and diluted with water to cause crystallization. The product consisted of yellow needle crystals which melted at 242° (decomp.). If the crystals were purified directly from acetone, they melted at 248°. The yield was 2.7 Gm. (64%).

Anal.—Calcd. for $C_{18}H_{14}N_4S$: N, 17.61%; S, 10.06%. Found: N, 17.11%; S, 9.58%.

6. The Hydrochloride of 5,5′-Diamino-8,8′-diquinolyl Sulfide.—The initial procedure was like the preparation of 5,5′-diamino-8,8′-diquinolyl sulfide. The tin complex was destroyed by adding 40% NaOH until the mixture was slightly acid; after stirring about half an hour the precipitate which formed was collected. The precipitate was purified by recrystallization from methanol. Orange needle crystals were obtained; m. p. 237.5–238.5°. The yield was 22%.

Anal.—Calcd. for $C_{18}H_{16}N_4SCl_2$: N, 14.32%; S, 8.18%. Found: N, 14.69%; S, 8.22%.

7. 5,5′-Diacetylamino-8,8′-diquinolyl Sulfide.—Dissolve 0.25 Gm. of 5,5′-diamino-8,8′-diquinolyl sulfide in 30 cc. of acetic anhydride, add 0.25 Gm. of anhydrous sodium acetate, and reflux on an oil bath for three hours. After cooling, the clear solution was poured into 100 cc. of ice water. Sodium carbonate solution was slowly added with continuous stirring; a gray precipitate appeared while the solution was still acid. The mixture was placed in an ice bath for about two hours, the precipitate was collected, dried, and purified by crystallization from a water-alcohol mixture. The gray crude crystals melted at 233–236°. Pure white crystals were obtained by recrystallization from a water-acetone mixture; m. p. 244°. The yield was 0.2 Gm. (63%).

Anal.—Calcd. for $C_{22}H_{18}N_4O_2S$: N, 13.93%; S, 7.96%. Found: N, 13.11%; S, 7.52%.

8. 5,5′-Diformylamido-8,8′-diquinolyl Sulfide.—One gram of 5,5′-diamino-8,8′-diquinolyl sulfide was dissolved in 20 cc. of formic acid (sp. gr. 1.2). The mixture was refluxed for two hours. After cooling, the reacted mixture was poured into 100 cc. of ice water. The red acidic solution was treated with a saturated sodium carbonate solution almost to the neutral point; an orange precipitate was obtained. The yield was 0.68 Gm. (58%), m. p. 277°. The product was purified further by recrystallization from acetone several times, m. p. 297° (decomp.).

Anal.—Calcd. for $C_{28}H_{14}N_4O_2S$: N, 8.56%; S, 14.97%. Found: N, 8.56%; S, 14.96%.

9. 5,5′-Bis-(*p*-acetamidobenzene Sulfonamido)-8,8′-Diquinolyl Sulfide.—One gram of 5,5′-diamino-8,8′-diquinolyl sulfide was dissolved in 20 cc. of dry pyridine, and 1.6 Gm. of *p*-acetamidobenzenesulfonylchloride and a small amount of copper-bronze catalyst were added. The mixture, which was contained in a flask attached to a condenser fitted with a calcium chloride tube, was heated on a steam bath for one hour. After cooling, the mixture was poured into 500 cc. of water and placed in an ice bath for about two hours. The precipitate was collected and recrystallized from acetone. When the substance first precipitated in the concentrated acetone solution, yellow crystals formed which changed to an orange crystalline substance after filtration. The yield was 0.9 Gm. (40%), m. p. 259°. When purified further by recrystallization from acetone, white crystals m. p. 263° (decomp.), were obtained.

Anal.—Calcd. for $C_{34}H_{28}N_6O_6S_3$: N, 11.80%; S, 13.48%. Found: N, 11.42; S, 12.86%.

SUMMARY

1. Five new quinoline derivatives possessing possible medicinal value have been synthesized, namely:

(*a*) 5,5′-Diamino-8,8′-diquinolyl sulfide.

(*b*) The hydrochloride of 5,5′-diamino-8,8′-diquinolyl sulfide.

(*c*) 5,5′-Diacetylamido - 8,8′ - diquinolyl sulfide.

(*d*) 5,5′ - Diformylamido-8,8′-diquinolyl sulfide.

(*e*) 5,5′-Bis-(*p*-acetamidobenzene sulfonamido)-8,8′-diquinolyl sulfide.

2. Unfortunately these quinoline derivatives have not been investigated for possible medicinal use; however, sufficient quantities have been prepared for bacteriological and pharmacological testing.

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Quantitative Determination of Saccharin*

By HAROLD ROSENBLUM and LEE MILDWORM

A convenient and rapid method for the assay of saccharin and saccharin sodium in tablets is described. The assay is effected by extracting the saccharin with a mixture of chloroform and ether and titrating the extracted saccharin with *N*/10 sodium hydroxide.

ALTHOUGH the U. S. P. XII method for the determination of saccharin in saccharin sodium tablets has been successfully employed, the analysts have always been aware that the method does not isolate the principal ingredient and that the results are dependent on the completeness of the hydrolysis of the NH group to NH_3. Furthermore, in order to insure that this method be workable, a test for ammonia in saccharin, saccharin sodium, and tablets of saccharin sodium is included in the Pharmacopœia. However, no assurance is given that nitrogen containing substances which slowly form ammonia during hydrolysis do not interfere with the assay.

EXPERIMENTAL

In order to circumvent interference from other NH_3 liberating compounds, the proposed method was investigated and found to be reliable and rapid for the determination of saccharin and saccharin sodium in tablet mixtures.

The chemical structure of saccharin is such as to give a strong acid reaction in aqueous solution. Attempts to titrate the solution using phenolphthalein T. S. as indicator gave excellent results. One-half gram of material accurately weighed and dissolved in 75 cc. of water by warming and cooling showed 99.5% saccharin by titration. Furthermore, saccharin was found to be appreciably soluble in a mixture of chloroform and ether (1:1). From these two established facts, the following procedure was evolved for tablet mixtures. Results are given in Table I with a comparison of values obtained with the U. S. P. XII procedure.

Place a sufficient number of tablets equivalent to 1 Gm. of saccharin in a 100-cc. volumetric flask, and add 50 cc. H_2O and 10 cc. *N* NaOH. Agitate until the tablets have disintegrated. Dilute to volume with water and filter through a dry filter into a dry flask, rejecting the first 20 cc. of filtrate. Transfer 25 cc. of the subsequent filtrate to a separatory funnel, acidify with dilute HCl, and extract the saccharin with 6 x 25-cc. portions of a mixture of chloroform and ether (1:1). Filter the combined extract through a pledget of cotton into a beaker and evaporate to dryness on a steam bath with the aid of a current of air. The residue obtained after evaporation of the solvents is taken up with 75 cc. of boiling water, the solution cooled to room temperature and titrated with *N*/10 NaOH, using phenolphthalein T. S. as indicator.

1 cc. of 0.1*N* NaOH = 0.01832 Gm. saccharin ($C_7H_5O_3NS$)

1 cc. of 0.1*N* NaOH = 0.02413 Gm. saccharin sodium ($C_7H_4O_3NSNa{\cdot}2H_2O$)

DISCUSSION

The interference of stearates in the proposed method was investigated and found to be negligible. In the initial filtration, it was found that practically all of the stearates are removed. Stearic acid if present reacts with sodium hydroxide in the cold too slowly to come through in the filtrate. Tablets containing stearic acid as much as 5 per cent of the weight of the tablet showed little or no stearate interference. The extracted saccharin evaporated to dryness and dried to constant weight at 90° showed approximately a 1 per cent higher assay value than by titration. Furthermore, filtration of the cooled aqueous solution of the extracted saccharin removes all insoluble organic acids that may be present.

TABLE I

Sample	Per Cent Based on Labeled Amount: Proposed Method	U. S. P. XII Method
Saccharin Powder	99.50%	99.4%
Special Granulation (50%)	102.2%	103.6%
Saccharin Tablets, 1/4 gr.	98.8%	99.6%
Saccharin Tablets, 1/2 gr.	97.2%	98.0%
	95.6%	95.6%
Saccharin Tablets, 1 gr.	96.0%	95.7%
Saccharin Sodium Tablets, 1 gr.	103.9%	103.2%

SUMMARY

A rapid, accurate determination of saccharin in saccharin sodium and in tablet mixtures is presented.

* Received June 14, 1946, from Premo Pharmaceutical Laboratories, Inc., New York 13, N. Y.

Evaluation of Totaquine in Treatment of Malaria in an Endemic Malarious Area*,†

By O. W. YEAGER,‡ R. F. REIDER,‡ and G. E. McDANIEL§

A PROGRAM for the purpose of evaluating the usefulness and toxicity of totaquine[1] as an agent in the treatment of naturally occurring malaria was instituted at the Malaria Field Research Station in Manning, S. C. The study which began on June 15, 1945, and extended through November, 1945, was carried on under field conditions in conjunction with other studies on the malarial disease and infections prevalent in the southern portion of Clarendon County, S. C.

Preliminary planning revealed the necessity for recognition of the usual limitations of a drug study carried out under field conditions. Therefore a complete appraisal of all factors involved in drug evaluations was not attempted. The primary objective of this study was simply to ascertain the clinical usefulness and relative toxicity of totaquine as compared with some other established and generally used antimalarial drug with a known degree of toxicity.

Quinine sulfate was chosen as the control drug because it is most frequently used by local physicians throughout the study area in the treatment of malaria, and is the chief ingredient of proprietary antimalarial drugs used by the people in self-medication for the treatment of symptoms alleged to be due to malaria. Quinine sulfate has been used as an antimalarial drug in the area for many years and is most often considered by the people to be the most acceptable drug.

The procedure of study consisted of the treatment at random of cases of clinical malaria with totaquine and quinine sulfate. The schedule of dosage was based on recommendations of the U. S. Army Medical Department (1). The adult therapeutic dose used for both drugs was 1 Gm. orally 3 times daily for two days and then 2 Gm. orally daily for five days. Proportionate dosages were utilized for children, depending upon weight and apparent age. No other drugs were prescribed. Each treated case was followed up to determine the relative acceptability of the two drugs, the occurrence of toxic symptoms, and to make a clinical appraisal of the relative effectiveness of the drugs as therapeutic antimalarial agents. Since this study was operated under natural field conditions, no extensive laboratory investigations were carried out.

The diagnosis of a case of malarial disease was based on clinical histories and physical examinations. A case of malarial disease was defined as a person presenting a history of an illness characterized by symptomatology typical of the disease complex known to be caused by plasmodial infections and physical findings typical of malarial pathology. Diagnoses of clinical malaria were made without regard to the blood smear findings.

The chief etiological agent of malarial disease observed in the Clarendon County area as demonstrated by routine monthly thick blood film surveys on the total population was *Plasmodium falciparum*. The cases of malaria were clinically of the simple, nonlocalizing, mild acute type. The symptomatology consisted of irregular, intermittent and remittent night fever, commonly followed by profuse sweating but rarely accompanied by manifest paroxysms. Other chief complaints were frontal and temperoparietal headaches, lumbar backaches, and generalized malaise. Physical findings revealed occasional splenomegaly not greater in size than the number-one type of Boyd's classification of splenomegaly (2) and moderate secondary anemia. All cases were Negroes and the age distributions in both

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† The studies on which this report is based were conducted cooperatively by the South Carolina State Board of Health and the U. S. Public Health Service, Office on Malaria Control in War Areas.

‡ U. S. Public Health Service, Atlanta, Ga.

§ Director, Division of Preventable Diseases, South Carolina State Board of Health.

[1] The totaquine used in this study was furnished through Defense Supplies Corporation and was manufactured by Merck and Co., Rahway, N. J.

the totaquine and quinine groups were uniform and ranged from six months through sixty years of age and over.

In all, 144 cases of malarial disease were treated, 102 with totaquine and 42 with quinine sulfate. Two cases (or 1.9 per cent) of those treated with totaquine and two cases (or 4.7 per cent) of those treated with quinine sulfate reported toxic symptoms of nausea and tinnitus which were severe enough to require discontinuance of treatment. With the exception of the 4 cases which showed moderate untoward reactions, all treated persons reported symptomatic improvement.

In general, totaquine was accepted and taken as readily as quinine sulfate. Both drugs were taken in preference to proprietary antimalarial drugs such as chill tonics.

SUMMARY

During a five-month period a controlled field study of the usefulness and relative toxicity of totaquine (Merck and Co.) as an antimalarial agent was compared with that of quinine sulfate. It was found that both drugs were accepted by the population with equal willingness and in preference to proprietary antimalarials. On the basis of observations in the field, totaquine was found to be about as effective as quinine sulfate in the symptomatic treatment of clinical malarial disease. Totaquine apparently causes no more untoward drug reactions when used under field conditions than quinine sulfate.

More careful evaluation of totaquine is recommended. This evaluation should be carried out where rigid controls can be set up and where necessary laboratory facilities are conveniently available.

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The Microscopic Identification of Demerol*

By GEORGE L. KEENAN†

Optical crystallographic data essential for the rapid and accurate microscopic identification of isonipecaine (Demerol) are reported.

DEMEROL is 4-phenyl-piperidine-4-carboxylic acid ethyl ester. It is a white crystalline substance which melts at 30° and is not very soluble in water. Its solutions are very alkaline. The hydrochloride is very soluble in water and in this form the substance is used medicinally. It is said to be a powerful analgesic and may become habit forming like morphine. Legally it is now classified as a narcotic, the name "isonipecaine" having been coined as a suitable designation instead of the trade name Demerol.

* Received July 26, 1946.
† Present address: Strongsville, Ohio, and Baldwin-Wallace College, Berea, Ohio.

The purpose of this paper is to place on record optical crystallographic data which are significant for Demerol hydrochloride thereby affording a rapid and accurate means for its microscopic identification. Data are also given which are characteristic of the crystalline complexes formed by Demerol hydrochloride with the reagents potassium iodide and sodium nitroprusside. (For convenience, unless otherwise qualified, in the following discussion Demerol shall refer to the hydrochloride.)

EXPERIMENTAL

Demerol is a white, crystalline powder, the habit being largely elongated, six-sided prisms. With crossed nicols the extinction is parallel and the sign of elongation positive. Many of the fragments do not extinguish sharply with crossed nicols, these showing one optic axis in the interference figure

when the substance is examined in convergent polarized light (crossed nicols). The significant refraction indices are: $\alpha = 1.545$, $\beta = 1.581$, $\gamma = 1.618$, all ± 0.002. All the indices are readily found on the substance.

Levine in a recent study (1) reported Demerol as giving significant crystalline complexes with several of the common alkaloidal reagents. As a double confirmatory test, the optical crystallographic examination of some of these complexes furnished information that is also useful analytically. This information is especially significant in the case of the crystalline precipitates formed respectively with potassium iodide and sodium nitroprusside.

Potassium iodide reagent (5 Gm. KI in 100 ml. of H_2O) produces fine colorless rods. These can be produced directly on an object slide, the excess reagent removed with small pieces of filter paper, and the precipitate allowed to dry at room temperature. When dry and examined with crossed nicols, the rods show inclined and parallel extinction, and the elongation is positive or negative. Many of the rods do not extinguish sharply with crossed nicols, showing flash biaxial interference figures in convergent polarized light (crossed nicols). The significant refractive indices of this crystalline complex are: $\alpha = 1.605$, $\beta = 1.625$ (most common of the indices), $\gamma = 1.653$, all ± 0.002.

With sodium nitroprusside, Demerol produces long, blade-like plates described and illustrated by Levine (1). He recommends a 5% aqueous solution of sodium nitroprusside as the test reagent. The writer has found that by dropping a small fragment of sodium nitroprusside (about 3 mg.) into the Demerol solution, just as satisfactory a precipitate is obtained. The blades which immediately form are characterized by a shingle-like surface. This characteristic of the surface is considered as quite diagnostic for the substance. After removing the excess liquid, the precipitate can be allowed to dry on the object slide at room temperature and subsequently examined with the polarizing microscope. With crossed nicols, these blades, frequently with obtuse terminations or hexagonal in outline, show inclined extinction and negative elongation. Three refractive indices characterize the substance and are as follows: $\alpha = 1.550$, $\beta = 1.582$, $\gamma = 1.615$, all ± 0.002.

It is also interesting to observe that picrolonic acid reagent (0.5% picrolonic acid in 50% alcohol) on being added to Demerol in a water-drop will at first cause a yellow cloudiness, followed by the gradual formation of spherulites of very fine needles. These aggregates are white and stand out in sharp contrast floating on the yellow liquid, growing to some size and readily observed even with the naked eye. The needles are too slender for microscopic study by the immersion method.

SUMMARY

The optical crystallographic properties of Demerol hydrochloride have been described and also those of two crystalline complexes formed with potassium iodide and sodium nitroprusside, respectively. The data for both of these addition products afford a doubly confirmatory microscopic test for Demerol. Attention is also called to the reaction of Demerol with picrolonic acid reagent.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Thiomorpholine
Chenodesoxycholic acid
Scymnol
Lysidine
Pheophytin
Purpuroxanthin
5,8-Dihydroxy-1,4-naphthaquinone
2,3-Dihydroxyanthraquinone
Alkannin
1-Amino-6-hydroxynaphthalene
o-Aminobenzaldehyde
α,α,-Dichloro-γ-valerolactone
d-Hydroxyglutamic acid
d-Isoleucine
l-Isoleucine
N-Methylphenobarbital
Pregnanediol, crystalline
Cevine
Pulegone
Sodium-*l*-tartrate
Sodium-*m*-tartrate
Homogentisic acid

Alabama's "Sheep Kill" Laurel, *Kalmia latifolia* L.—A Preliminary Study*

By WILLARD J. HADLEY† and HUGH H. HADEN, JR.‡

"Sheep kill" laurel, or "mountain laurel," a bane to cattle raising in Alabama, was identified as *Kalmia latifolia* L. Half mature leaves were ground and extracted with a series of solvents. Glycosides were present in all extracts prepared with organic solvents. Extractives obtained with isopropyl alcohol were hydrolyzed with hydrochloric acid and aglycone materials separated according to their differences in solubilities. These appeared to be catechol-4-catechuic acid and *cis* and *trans* isomers of *d*-catechol. A hydroxy flavane and a hydroxy chromane were indicated and both appeared to be affected by acid in the same manner as flavones.

SHEEP and cattle have been observed to suffer nausea, vertigo, and progressive paralysis from nibbling the foliage of a laurel which frequents fence rows in Alabama. These animals show little effect from feeding on the young plant, and deer and goats appear little affected on eating the laurel at any stage of its development. Honey that is made by bees which have access to laurel, rhododendron, and others of the Heath family is regarded as unsafe for human food, but the bees store and consume it with safety. Household decoctions of laurel leaves have been given in diarrhea and employed as lotions in skin diseases. Preparations of the leaves have even acquired reputations, in some sections, as palliatives in syphilis.

At the same time that the effects of this plant came to our attention, botanists at Tuskegee Institute and the George Washington Carver Foundation began a systematic identification of this bane to cattle growers. These investigators established it as *Kalmia latifolia* L., popularly called "mountain laurel," "broad-leaf laurel," "calico bush," and "spoonwood" (1, 2). They volunteered to supply us with quantities sufficient for preliminary chemical examination, and collected for this study about 10 pounds of the leaves in Marion County, Ala., in the month of June, a time when the plant is of nearly normal size but scarcely mature.

In this first crop, taken before the laurel had stored much of its toxic constituents, we have found minute amounts of 2 glycosides. The aglycone portions of these appear to be protocatechuic acid and *d*-catechol (*cis* and *trans* isomers), known compounds (3–5), and the sugars, fructose, glucose, xylose, and arabinose. Chemical confirmation is incomplete. Photomicrographs of the aglycone materials were obtained. No report has been found in the literature of pharmacologic testing of these compounds. We have thus far been unable to isolate amounts adequate for thorough chemical study or for physiological examination, and will resume the investigation when older laurel is available. Director R. W. Brown, of the George Washington Carver Foundation, and Professor H. J. Romm, of Tuskegee Institute, are collecting for us larger quantities of the leaves, taken at a more advanced stage of the plant's growth. The extractive principles so far obtained have appeared easily altered chemically. We shall, in repeating certain parts of this work, make some significant adaptations in procedure in the hope of overcoming losses at present sustained.

A toxic extractive was obtained from *Kalmia latifolia* by Lasché, in 1889, and named by him "andromedotoxin" (6). Lasché and DeZaayer found "andromedotoxin" nonnitrogenous. Their reported findings suggest that it was a glycoside (7). It is evident that "andromedotoxin" was a mixture. The melting points of their

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material were reported variously. The present study was undertaken for the purpose of isolation and identification of "andromedotoxin" or whatever poisonous agent is contained in Alabama's "Sheep Kill" laurel. Future work will include observation of the physiological properties of the principles we have found indicated in this "mountain laurel."

EXPERIMENTAL

Air-dried leaves of the laurel were ground to number 20 powder.

The "drug constants" of the United States Pharmacopœia were found. The averages of these were as follows:

"Characteristic" or "Constant"	%
Moisture	6.70
Total ash	3.43
Acid-insoluble ash[1]	0.20
Ligroin-soluble extractives	4.54
Ether-soluble extractives	10.30
Alcohol-soluble extractives	30.80
Hydroalcohol-soluble extractives	5.67
Water-soluble extractives	17.25

The drug was extracted, in Soxhlet apparatus, with 5 solvents, successively employed; viz., ligroin, ether, chloroform, alcohol, water. None of these extracts gave indications of the presence of alkaloids with the usual precipitants.

From the ligroin extract a small amount of paraffin-type wax was obtained, by chilling an 80% alcohol mixture. It melted indefinitely, and did not yield to fractionation by distillation *in vacuo*. The ligroin extract was observed to contain, also, some fixed oil, which was associated with resins that were made up of high molecular weight acids. The saponification number of the oily mixture was 126 (8). Sterol tests (9, 10) were faint. Glycoside tests (11) were clear but weak.

The ether extract yielded to cold water some catechol-type tannins (green in ferric chloride solution), some glycosidic material (11), and sugars. These latter formed addition products with 2,4-dinitrophenylhydrazine but were incapable of restoring color to Schiff's reagent. This was evidence that the sugars were ketones.

The chloroform extract gave tests which paralleled those of the ether extract. This extract also bore difficultly soluble, fat-like material, indifferent toward all reagents and judged to be resenes.

The alcohol extract gave the indications of glycosides, of catechol tannins and of ketonic sugars above, and yielded extractive material which was insoluble in alkali bicarbonate but soluble in alkali hydroxide, confirming phenols. An osazone formed in two minutes, the reported time for fructosazone (12).

[1] Found to contain calcium carbonate and ferrous sulfide.

Molisch tests (11) and Fehling's tests (13) were both positive on an acetone extract of a fresh sample, but the Molisch tests were negative on the same extract after the extract had been warmed with 5% HCl. These observations lent evidence that the glycosides contained in the drug were easily hydrolyzable.

The water extract, fifth in the series, above, gave Molisch tests and catechol tannin tests and yielded an osazone in five to fifteen minutes (clearly a mixture). The time factor, here, suggested glucose, xylose, and arabinose sugars (12). These are all aldehydes, and tests for carbonyl compounds in the ether extract, above, and the time of formation of the osazone from alcohol extractives, above, indicated ketonic sugars, specifically fructose. But glucose, xylose, or arabinose could conceivably be only a portion of the carbohydrate part of a glycoside, between the aglycone and a fructose end unit. The carbohydrate would be cleaved more easily than the aglycone would be liberated, and, thus, while free fructose would be expected in the extracts obtained with organic solvents, above, extraction of the drug with water would be accompanied by liberation of the aglycone from the immediately adjacent portion of the carbohydrate. This would give in the water extract these aldehydic sugars, which were not indicated in the ether, chloroform, and alcohol extracts. No characteristic physical form could be observed microscopically in the mixture of osazones.

Isopropyl Alcohol Extract.—One kilogram of drug was extracted thoroughly, in Soxhlet apparatus, with isopropyl alcohol. The extract was acidified with HCl, then evaporated at moderate heat almost to dryness, and, finally, stirred into 5 L. of cold water. A flocculent, grayish precipitate, consisting perhaps of aglycone substances and secondary glycosides, was recovered by centrifuging and washed with an ether-acetic acid mixture. These washings yielded small amounts of two kinds of crystals, in a viscous, amber-colored matrix, the one filamentous and lacy and the other in elongated needles (10×). The mixture was triturated with sand and extracted with chloroform. The chloroform extract yielded a few of the needle-shaped crystals but none of the other crystals. The amount obtained was too small to permit recrystallization. The crude material melted at 190–210°, with decomposition. It produced a green coloration with ferric chloride, then red with sodium bicarbonate. These tests, together with the melting temperature observed, suggested catechol-4-carboxylic acid, called also protocatechuic acid and veratric acid (3). A photograph of these crystals, in their dark, viscous matrix, is given in Fig. 1 (*A*) (100×).

When a mixture of the two kinds of crystalline material was extracted with, first, cold glacial acetic acid and, second, chloroform, the former of these extracts was found to contain a small amount of the lacy crystalline material, above, and the latter extract yielded a few of the needle-shaped crystals.

The former deposit was laboriously separated with a teasing needle, under a magnifying glass, into what appeared as main threads and what appeared as filler threads. These "main threads" melted, without recrystallization, at 177–180°. The "filler threads" melted, without recrystallization, at 240–245°. Both produced a green coloration with ferric chloride. Both were found soluble in water, alcohol, ether, and dilute alkali hydroxide, but not in dilute alkali bicarbonate. These tests, together with the melting temperatures observed, suggested the two geometric isomers of *d*-catechol (4, 5). A photograph of this crystalline material, in its viscous, amber-colored matrix, is given in Figure 1 (*B*) (100×).

A B

FIGURE 1

The last of the drug on hand, somewhat less than 1 Kg., was percolated with isopropyl alcohol. The percolate was caught and distributed in a mixture of purified sand and gravel. The solvent was removed by distillation, and the residue was steam distilled. The distillate contained volatile oil and a small amount of crystalline material which satisfied the tests for catechol-4-carboxylic acid. This material was rapidly discolored by exposure to air, precluding recovery. The residue of steam distillation was extracted with glacial acetic acid. This extract was distributed on sand and gravel and evaporated to dryness and the absence of acetic acid odor. The residue was then treated with boiling water. The water solution, dark brown, gave tests for the presence of catechol-4-carboxylic acid, but yielded no crystalline substance. Instead, fairly abundant brown-black, amorphous material settled out. It was apparent that the hot acid produced changes in the flavanol and the chromanol which gave deeply colored, amorphous compounds. The acid could be expected to produce ring-opening in either of these, giving reduced chalcones that gave cations which conferred color (14).

SUMMARY

Alabama's "sheep kill" laurel, which was identified by collaborators as *Kalmia latifolia* L., was found to store two glycosides, as it approached maturity, but gave no indications of alkaloids. The writers hypothesize that a mixture of these glycosides was Lasché and DeZaayer's "andromedotoxin." Small quantities of aglycone materials were obtained in impure state. The preliminary chemical study and the photomicrographs obtained suggested catechol-4-carboxylic acid and both the geometric isomers of *d*-catechol. Glucose, xylose, arabinose, and fructose were the indicated sugars.

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The Thiamine Content of Herbs and Medicinal Plants*

By ALEXANDER S. CHAIKELIS†

By means of a photofluorometric assay technique, eliminating the elution phase, devised to investigate the thiamine content of plant material ranging from rhizomes and roots to barks, leaves, and flower tops, the thiamine concentration in herbs and medicinal plants was determined. A spot check analysis of 41 genera belonging to 20 distinct and separate botanical families showed a thiamine range from 125 to 2880 micrograms per 100 Gm. of dried substance. This vitamin B_1 content was postulated as a possible modus operandi of the many infusions and decoctions of medicinal herbs prescribed in cases of anorexia, dyspepsia, general debility, and nervousness.

FOR MANY CENTURIES the use of plants in the alleviation and cure of bodily ills received wide attention but little official sanction until in the reign of King Henry VIII Parliament enacted what is now popularly known in England, "The Herbalists' Charter" (1).

Healers, like Nicholas Culpepper (1616–1654) in England and Samuel Thompson (1769–1843) in America, did much to advance the study and practice of herbalism. Apart from the claims made by the herbalists, the use of herbs as sources of drugs, like those described in articles and books on pharmacognosy and/or materia medica, has done much to justify their usefulness in medicine (2–7).

With our present knowledge of the various sources of vitamins, can it be that the cumulative beneficial results produced by the use of herbs, exclusive of their medicinal properties, might be the effects of increased vitamin intake? To obtain information along this line of thought, it was decided to make a spot check on some herbs and other plants, emphasis to be on those which were and are, at present, described as possessing tonic, stomachic, and stimulant actions in cases of dyspepsia, constipation, general debility, and nervousness. Since all higher plants are able to not only synthesize thiamine but to excrete it through the roots, it seemed logical to investigate the existence and quantity of thiamine in these and other parts of the dried plants that had recognized medicinal value.

Samples of the herbs and medicinal plants were purchased in the dried state from a reliable distributor of such material. The plants so bought represented 41 genera belonging to 20 separate botanical families. This number of specimens was considered to be an adequate sampling of the entire group of medicinals.

EXPERIMENTAL

Based on the idea of procedure for thiochrome determination described by Hennessy (8) and the digestion method of plant material to release the thiamine in the free state as well as the phosphorylated form (cocarboxylase) suggested by Platt and Block (9), the thiamine content of the plant material was analyzed by the following method devised to fit the demands of the material under investigation and the requirement of a quick, reliable technique.

A. Reagents Required.—The many reagents needed to effectively carry out the thiochrome determinations included those listed in the U. S. P., those enumerated by Platt and Block (9), and those, such as the needed *standard solutions*, described in (10).

The digesting enzymes papain and clarase before use were treated in the following manner: A 5-Gm. batch of each enzyme is suspended in 50 cc. distilled water and shaken thoroughly. To this suspension is added 50 cc. isobutanol, and the mixture again shaken in a separatory funnel. Discard the supernatant isobutanol fraction, repeating the process until the isobutanol portion when oxidized with NaOH and $K_3Fe(CN)_6$ shows no evidence of thiochrome when examined in a photofluorometer.

The aqueous fraction is then treated with ethanol to precipitate the enzymes, filtered, and the residue dried *in vacuo*. The dry amorphous powder is then ready for use in the digestion phase of the treatment section of the procedure.

* Received July 9, 1946, from the Biology Department, The City College, and the Department of Industrial Hygiene, School of Public Health, Columbia University, New York City.

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TABLE I.—COMPOSITE SUMMARY OF THIAMINE CONTENT IN VARIOUS DRIED HERBS AND OTHER MEDICINAL PLANTS

Common Name	Genus and Species	Family	Parts Used	Av. Dose in Gm.	Thiamine in γ/Gm.	Thiamine in γ/Dose	Thiamine in γ/100 Gm.	Action and Method of Administration
Agrimony	*Agrimony eupatorium* Linn.	Rosaceae	Entire herb	4	2.355	9.42	235.5	Tonic; as an infusion: 1 oz. to 1 pt. water
Angostura	*Galipea officinalis*	Rutaceae	Bark	2	11.286	22.56	1128.6	Bitter tonic; as above, 2 fl. oz. t. i. d.
Balmony	*Chelone glabra* Linn.	Scrophulariaceae	Leaves	4	6.594	26.37	659.4	Tonic, for constipation; as above, 2 fl. oz. t. i. d.
Barberry	*Berberis Aquifolium* (Pursh.)	Berberidaceae	Root bark	2	10.038	20.07	1003.8	Bitter tonic; powdered bark, 20 gr. t. i. d.
Bay Laurel	*Laurus nobilis* Linn.	Lauraceae	Leaves	..	7.928	....	792.8	Flavoring: spice for meats, poultry, meat sauces
Black Alder	*Ilex verticillata* Linn.	Aquifoliaceae	Bark	2	22.464	44.94	2246.4	Tonic; as infusion, 2 fl. oz. q. i. d.
Blessed Thistle	*Cnicus benedictus* (Goetn.)	Compositae	Entire herb	4	5.879	23.52	587.9	Tonic; as above
Boneset	*Eupatorium perfoliatum* Linn.	Compositae	Leaves	2	5.378	10.74	537.8	Tonic; as above, 2 fl. oz. q. i. d. Served cold
Buchu	*Barosma betulina* Bartling and Wendl.	Rutaceae	Leaves	2	19.656	39.30	1965.6	Stimulant; as above, 2 fl. oz. q. i. d.
Bugleweed	*Lycopus uniflorus* Linn.	Labiatae	Entire herb	4	2.960	11.85	296.0	Calmative; as above
Calamus	*Acorus Calamus* Linn.	Araceae	Rhizome and roots	3	5.338	16.02	533.8	Aromatic bitter; as above
Caraway	*Carum Carvi* Linn.	Umbelliferae	Seeds	1	7.222	7.23	722.2	Stimulant, carminative; 2 fl. oz. t. i. d. as an infusion
Cascarilla	*Croton Eluteria* Bennett	Euphorbiaceae	Bark	2	8.763	17.52	876.3	Bitter; as infusion, a 2-fl. oz. dose t. i. d.
Catnip	*Nepeta cataria* Linn.	Labiatae	Leaves	4	5.600	22.38	560.0	Tonic; as infusion, 2 fl. oz. q. i. d.
Celery	*Apium graveolens* Linn.	Umbelliferae	Seeds	2	7.928	15.81	792.8	Stimulant; as infusion, 2 fl. oz. t. i. d.
Chamomile	*Matricaria Chamomilla* Linn.	Compositae	Flower tops	15	11.542	173.13	1154.2	Bitter; as infusion, 2 fl. oz. q. i. d.
Cherry Tree	*Prunus serotina* Linn.	Rosaceae	Leaves	2	18.720	37.38	1872.0	Bitter tonic; as infusion, 2 fl. oz. t. i. d.
Condurango	*Marsdenia Condurango* Reichenbach f.	Asclepiadaceae	Bark	4	10.340	41.25	1034.0	Bitter; as infusion, as above
Dandelion	*Taraxacum officinale* Weber	Compositae	Rhizome and roots	4	15.774	63.09	1577.4	Simple bitter; as infusion, 2 fl. oz. q. i. d.
Dogwood (Jamaica)	*Piscidia erythrina* (Jacques)	Leguminosae	Root bark	2	5.652	11.31	565.2	Bitter tonic; as infusion, 2 fl. oz. t. i. d.
Feverfew	*Chrysanthemum Parthenium* Bernh.	Compositae	Entire herb	2	5.024	10.05	502.4	Calmative in hysteria; as infusion, 2 fl. oz. t. i. d.
Garden Sage	*Salvia officinalis* Linn.	Labiatae	Leaves	4	8.544	34.17	854.4	Carminative; as infusion for dyspepsia, as above
Gentian	*Gentiana lutea* Linn.	Gentianaceae	Rhizome and roots	1	4.774	4.77	477.4	Simple bitter; as infusion, 2 fl. oz. q. i. d.
Ghost Root	*Angelica archangelica* Linn.	Umbelliferae	Rhizome and roots	2	6.832	13.68	683.2	Bitter; as above
Ground Ivy	*Nepeta hederacea* Linn.	Labiatae	Entire herb	4	6.029	24.12	602.9	Tonic; as infusion in dyspepsia, as above
Horseradish	*Cochlearia armoracia* Linn.	Cruciferae	Rhizome and roots	4	5.024	20.10	502.4	Stomachic; as infusion, 2 fl. oz. t. i. d.
Moonseed	*Menispermum canadense* Linn.	Menispermaceae	Rhizome and roots	1	8.653	8.64	865.3	Simple bitter; as above, 2 fl. oz. t. i. d.
Musk Root	*Ferula Sumbul* Hooker filius	Umbelliferae	Rhizome and roots	2	6.537	13.08	653.7	Calmative; as an extract in 8-gr. doses
Parsley	*Carum petroselinum* (Benth.)	Umbelliferae	Leaves	..	4.396	....	439.6	Flavoring; used ad libitum
Parsley	*Carum petroselinum* (Benth.)	Umbelliferae	Rhizome and roots	3	1.256	3.78	125.6	Calmative; as infusion, 2 fl. oz. t. i. d.
Prickly Ash	*Xanthoxylum americanum* Miller	Rutaceae	Bark	2	28.800	57.60	2880.0	Stimulant; as infusion, 2 fl. oz. q. i. d.
Quassia	*Picrasma excelsa* Panchon	Simarubaceae	Heartwood	1/2	18.386	9.18	1838.6	Bitter tonic; 0.5 cc. as fluidextract
Scullcap	*Scutellaria lateriflora* Linn.	Labiatae	Entire herb	1	6.908	6.90	690.8	Tonic; as infusion, 4 fl. oz. q. i.d.
Snakeroot (Can.)	*Asarum canadense* Linn.	Aristolochiaceae	Rhizome and roots	2	4.384	8.76	438.4	Aromatic bitter; as infusion, 2 fl. oz. t. i. d.
Snakeroot (Va.)	*Aristolochia serpentaria* Linn.	Aristolochiaceae	Rhizome and roots	1	3.657	3.66	365.7	Aromatic bitter; as infusion, as above
Southernwood	*Artemisia abrotanum* Linn.	Compositae	Entire herb	4	2.000	8.00	200.0	Emmenagogue; as infusion, 2 fl. oz. q. i. d.
Strawberry (wild)	*Fragaria virginiana* Linn.	Rosaceae	Leaves	4	2.800	11.19	280.0	Bitter tonic; as infusion, 2 fl. oz. t. i. d.
Tansy	*Tanacetum vulgare* Linn.	Compositae	Entire herb	2	5.338	10.68	533.8	Stimulant, antispasmodic; as infusion, as above
Thyme (common)	*Thymus vulgaris* Linn.	Labiatae	Leaves	4	5.024	20.10	502.4	Stimulant, antispasmodic; as above
White Ash	*Fraxinus americana* Linn.	Oleaceae	Bark	2	20.585	41.16	2058.5	Bitter tonic; as infusion, 2 fl. oz. t. i. d.
White Cinnamon	*Cinnamomum Cassia* (Blume)	Lauraceae	Bark	2	4.270	8.54	427.0	Aromatic bitter; as above
Yellow Dock	*Rumex crispus* Linn.	Aristolochiaceae	Rhizome and roots	4	10.261	41.04	1026.1	Stomachic, tonic; as above

B. Treatment of the Substance to Be Analyzed.—1. Place 10 Gm. of the substance, dried and finely ground, in a 250-cc. flask.

2. Add 90 cc. $N/10$ H_2SO_4, mix thoroughly, and then add 0.2 Gm.-batches each of the purified papain and clarase.

3. Now add 10 cc. of the $M/10$ sodium acetate-acetic acid buffer solution. Mix well (check *p*H with bromcresol green so that it is 4.0; correct with either 10% HCl or 10% NaOH).

4. Stopper flask and heat the mixture at 40–45° for from sixteen to eighteen hours.

5. Remove flask, add 0.2 cc. glacial acetic acid, and heat mixture to 80° to coagulate the proteins.

6. Cool flask to room temperature; centrifuge a 20-cc. portion, and save the clear supernatant aqueous layer for the analysis.

7. Oxidation (by means of alkaline ferricyanide) of thiamine to thiochrome, which is extracted by isobutanol, is followed by measurement of the fluorescence in ultraviolet light of the thiochrome formed. The thiamine pyrophosphate also forms a fluorescent thiochrome, which is not, however, soluble in isobutanol—hence one reason for the enzyme treatment to hydrolyze the thiamine pyrophosphate to thiamine.

8. With a Coleman Universal Spectrophotometer #11 adapted with an ultraviolet illuminator and the requisite filters for thiamine study, determine the degree of fluorescence using the substitute working standard of quinine sulfate (3 gamma per cc.). See details in the direction booklet (11).

RESULTS

The values for the vitamin B_1 content of the 41 genera of herbaceous medicinal plants are represented in Table I. The thiamine, in micrograms per gram of dried substance, as listed in the table represents the *mean* value of three distinct and separate samples of each plant studied.

Of the total number of plants studied in this spot check, it can be seen by reference to Table I that a group of seven medicinal plants contain thiamine in excess of 1500 γ/100 Gm. of substance. These seven plants arranged in order of the highest thiamine content are: Prickly Ash (*Xanthoxylum americanum* Miller), Black Alder (*Ilex verticillata* Linn.), White Ash (*Fraxinus americana* Linn.), Buchu (*Barosma betulina* Bartling & Wendland), Cherry (*Prunus serotina* Linn.), Quassia (*Picrasma excelsa* Panchon), and Dandelion (*Taraxacum officinale* Weber).

On the other hand, five genera show a thiamine content of less than 300 γ/100 Gm. of dried substance. Arranged in order from the lowest to the highest concentration these five herbs are: Parsley (root) (*Petroselinum sativum* Hoffman), Southernwood (*Artemisia abrotanum* Linn.), Agrimony (*Agrimony eupatorium* Linn.), Strawberry (wild) (*Fragaria virginiana* Linn.), and Bugleweed (*Lycopus uniflorus* Linn.). The remaining herbs in the series range from 365–1154 γ/100 Gm. of substance.

DISCUSSION

Many factors influence the effectiveness with which a plant synthesizes vitamins. Therefore the values reported in this paper cannot be considered as constant but merely as indicative of the concentration of thiamine contrary to the claim made that leaves, for instance, belonging to higher plants regardless of the botanical family source contain a constant thiamine concentration equal to 75 micrograms per 100 Gm.

It is an accepted fact that animals (including man) require a daily intake of thiamine because this vitamin is not stored in the organism although, as Westerbrink (12) reported, relatively higher concentrations are found in the liver, kidneys, heart, muscle, and brain than in the blood stream. The animal organism absorbs only as much thiamine as is needed for the time being, all excess is excreted and to a small extent destroyed in the body.

The physiological action of thiamine in the animal organism in the form of its pyrophosphoric acid ester is intimately concerned with carbohydrate, fat, and water metabolism. Thus, glycogen, the specific animal carbohydrate, requires the presence of this vitamin to be properly metabolized. The fat metabolism is influenced by vitamin B_1 only in so far as the synthesis of fat from carbohydrate is concerned. The water metabolism in the animal organism seems connected with the action of this vitamin, probably through the carbohydrate metabolism. Edema and water imbibitions in the heart and in other organs are symptoms of thiamine inadequacy. Vitamin B_1 deficiency affects, first of all, the emotions and the tonus of the nervous system, for this substance takes part in the conduction of nerve impulses as shown by Minz (13) that acetylcholine and vitamin B_1 are liberated during impulse conduction.

Further symptoms of thiamine deficiency are loss of appetite (anorexia), lowered physical endurance associated with unusual susceptibility to fatigue, gastrointestinal

disturbances (dyspepsia, constipation), muscular weakness, and a decrease of the blood pressure.

Attention is called to the fact that it is precisely for such discomforts that many herb infusions and decoctions, known as "tonics," have been prescribed. The indications are that these prescriptions materially benefited the ailing individual, at least in a small measure, by providing him with readily absorbable thiamine and its phosphorylated form at a time when his system was in great need of these items.

SUMMARY

1. The thiamine content of a spot check analysis of 41 genera of herbs and medicinal plants was determined by means of the photofluorometric technique.

2. A photofluorometric assay technique was devised employing the suggestions of Hennessy (8) and those of Platt and Block (9).

3. These 41 genera of herbs and medicinal plants belonging to 20 separate botanical families showed a thiamine content ranging from 125–2880 micrograms per 100 Gm. of dried substance.

4. The rational basis for the use of herb infusions and decoctions in the form of "tonics" is suggested on the basis of the physiological action of thiamine in the animal organism.

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Persistence of Penicillin in Saliva After Local Oral Application*

By MILTON GJELHAUG LEVINE and ROBERT E. HOYT

Penicillin for local application to the mouth area was administered in a chicle base chewing gum, in flavored paraffin, and in a mouthwash. The retention time of penicillin in the saliva for each of these dosage forms with varying concentrations of the drug is recorded in this report.

THE USE of penicillin in the buccal cavity has been suggested in hemolytic streptococcus infections, tonsillitis, Vincent's disease (acute ulcerative gingivostomatitis), Ludwig's angina, for lesions of the oral cavity involving loss of surface, and for prophylactic use in operative procedures of the mouth. Of all these, the most convincing therapeutic evidence has been adduced for the use of local preparations in Vincent's angina.

Our own experiences and those reported by other investigators (1, 2, 9, 10) indicate that in most cases Vincent's disease may be cured in from twenty-four to forty-eight hours by the local application of penicillin. The results for other infections so far have been inconclusive, although preliminary reports (2–5) indicate that Group A streptococci may be reduced in number and even eliminated by local applications of penicillin. However, more satisfactory results may be obtained by parenteral administration of the drug. Parenteral administration of penicillin has also proved to be of value in Ludwig's angina (6, 7).

The treatment of acute ulcerative stoma-

* Received Aug. 12, 1946, from the Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, Calif.

titis by local application of penicillin is obviously preferable to parenteral administration, since most cases are ambulatory, seen either by the physician or dentist. Two preparations have been suggested for this purpose: the pastille (2), and the mouthwash (1). Gregory and Lang (2) incorporated 500 units of penicillin into a pastille. Penicillin was said to be present in the saliva up to thirty minutes after removal of the pastille although no unitage was mentioned. The mouthwash suggested by Schmidt and Horwitz (1) contained 200 units of penicillin per cubic centimeter of normal saline. The mouth was washed by swishing one mouthful of the solution around the mouth once every four hours. Again, no figures were reported on persistence of the drug in the saliva. Other preparations have been suggested: Strock (8) has reported having patients hold a solution containing 5000 units per cubic centimeter in the mouth for periods up to one hour. Denny and co-workers (9) swabbed a solution containing 250 units per cubic centimeter on the Vincent's ulcers four times daily. In both instances, the methods were cumbersome and the assay data supplied were inadequate.

To supply more complete data on the persistence of penicillin in the mouth after local application the following experimental study was undertaken.

EXPERIMENTAL

For local application to the mouth area, penicillin was administered in chewing gum, in a mouthwash, and in a flavored paraffin base. The gum contained 400 units of penicillin per stick (1 Gm. of chicle base flavored with saccharin and wrapped in lead foil). The gum was chewed during the entire period of observation. The mouthwash was prepared by combining penicillin with dibasic and monobasic sodium phosphate to give a *p*H of 6. This tablet was dissolved in 20 cc. of water; the whole volume was then swished around in the mouth for one minute and expectorated.

The third medication consisted of calcium penicillin in flavored paraffin and was chewed during the entire observation period.

Assays of penicillin in saliva (made by the Oxford cup technique) were employed as an index of persistence of the drug in the mouth. The results are reported in Table I. The sign > indicates greater than, the sign < indicates less than the number of units reported. Each figure represents an average of at least four observations on four different subjects.

TABLE I.—PERSISTENCY OF PENICILLIN IN SALIVA IN UNITS PER CC. OF SALIVA

Time, Hr.	Gum 400 Units Total	Paraffin, 10,000 Units Total	Paraffin, 20,000 Units Total	Paraffin, 40,000 Units Total	Mouthwash[a] 200 Units/Cc.	Mouthwash[a] 5000 Units/Cc.	Mouthwash[a] 10,000 Units/Cc.
1/2	>1	>1	>1	>1	0.5	>1	>1
1	>1	>1	>1	>1	0.25	>1	>1
1 1/2	1	>1	>1	>1	<0.25	>1	>1
2	0.5	1	>1	>1	<0.25	>1	>1
2 1/2	0.25	1	>1	>1	0	>1	>1
3	<0.25	1	>1	>1	....	>1	>1
3 1/2	....	<0.25	0.5	>1	....	0.25	0.5
4	....	0	0.5	>1	....	<0.25	0.5

[a] A total of 20 cc. of mouthwash was used in each case.

DISCUSSION

The persistence of penicillin in the mouth for periods up to four hours in length depends on the amount of penicillin involved regardless of the preparation. Of special interest is the prolonged presence in the mouth of penicillin after washing with the medicated solution for only one minute. The choice of an oral preparation will depend on the particular circumstance. Where the patient can chew for long periods, the gum or paraffin type is preferable, since the total number of units required for a continuous high level is less than with the mouthwash. However, where the patient cannot chew the mouthwash is desirable.

It must be emphasized that if a mouthwash is used, no food or liquid should be employed since these will undoubtedly dilute the penicillin remaining in the mouth.

It is difficult to set a therapeutic level for penicillin in the mouth since no chemotherapeutic investigation has been carried out on the controversial organism thought to be the cause of Vincent's disease. However, since 0.3 of a unit of penicillin (10) is the upper limit reported to be needed for bacteriostasis of susceptible bacteria in plasma, such a figure must arbitrarily be used in interpreting the data herein presented for saliva.

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The Interaction of Sympathomimetic Pressor Amines: Inversion of the Vonedrine Pressor Action by Paredrine*

By RAYMOND P. AHLQUIST†

The presence of Paredrine [β(*p*-hydroxyphenyl)-isopropylmethylamine] in the anesthetized dog causes the pressor action of Vonedrine (β-phenyl-*n*-propylmethylamine) to become inverted to a depressor action. Correlation of the arterial pressure and the rate of blood flow in the hind leg or head shows that this depressor action is due to peripheral vasodilation. Repeated doses of Vonedrine alone produce a diminishing pressor response but no depressor action. Other amines, including epinephrine, ephedrine, tyramine, Sympatol, and Propadrine, do not invert Vonedrine and are themselves not inverted by Paredrine. Benzylimidazoline, a sympathomimetic antipressor agent, does not invert Vonedrine. The findings in this study indicate that the inversion of the Vonedrine pressor response by Paredrine may be an example of true reversal of action and not an unmasking of a normally present response.

THE CARDIOVASCULAR response to a sympathomimetic pressor amine is always modified by the presence of a previous dose of the same or another amine. The pressor response may be augmented or diminished or converted completely or partially to a depressor response. The pressor action of epinephrine is usually increased by the presence of ephedrine although in some cases it may be diminished (1). Lewis (2) has recently shown that several N-substituted hexylamines potentiate the pressor action of epinephrine, ephedrine, phenylethylamine, and cyclohexylethylmethylamine. A diminishing pressor response to repeated administrations (tachyphylaxis) occurs with a large number of sympathomimetic amines. Cross tachyphylaxis can also occur. Ephedrine has been shown to prevent the pressor action of some of the aliphatic amines thus allowing a depressor action to appear (3). Hambourger and Jamieson (4) showed that β-(*p*-ethylphenyl) ethylmethylamine reversed the pressor action of phenylethylamine and other similar compounds.

This report concerns another example of inversion of the pressor response; the pressor action of Vonedrine (β-phenyl-*n*-propylmethylamine) is converted to a depressor action by the presence of Paredrine (β-(*p*-hydroxyphenyl) isopropylmethylamine). Paredrine has an action similar in most respects to that of ephedrine (5, 6), lacking, however, the marked central stimulant action. Tachyphylaxis to the pressor response develops if the doses are rapidly re-

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peated (7). Vonedrine, a volatile amine which has been proposed as a nasal vasoconstrictor, also has an action similar to ephedrine (8). Tachyphylaxis to the pressor action of this drug has not been described.

EXPERIMENTAL

Dogs lightly anesthetized with morphine and ether were used throughout. Blood pressure was recorded from the carotid or femoral artery by means of a mercury manometer or a Hamilton manometer (9). Blood flow was measured in the hind leg or head by inserting a Gregg-Shipley Rotameter (10) into the appropriate artery following the administration of heparin. The height of the float in the Rotameter was recorded on the kymograph by an electro-mechanical system. Atropine (0.5 mg. per Kg.) was administered to the animals to abolish the vagal reflex and other postganglionic cholinergic effects. The amines were used in the form of their water-soluble salts and all doses are given on a milligram per kilogram basis.

after Paredrine producing a marked fall in blood pressure associated with active peripheral vasodilation. If the dose of Paredrine was equal milligram for milligram with the dose of Vonedrine no pressor action of the Vonedrine was apparent. If, however, the dose of Paredrine was one-half to one-third that of the Vonedrine then Vonedrine produced a slight rise in pressure after the primary fall. With 1 mg. of Paredrine this reversal action persisted from one to two hours. Repeated doses of Vonedrine alone produced a diminishing pressor response (tachyphylaxis) but in no case did a depressor action become apparent.

At least two distinct mechanisms should be involved in this reversal of Vonedrine by Paredrine since the pressor action of Vonedrine is due both to cardiac stimulation and peripheral vasoconstriction. That Paredrine converts the vasoconstrictor action of Vonedrine to vasodilation is apparent in the blood flow studies. Vonedrine normally increased the heart rate in the atropinized dog during the pressor action but after Paredrine this increase in heart rate did not occur. This may be taken as evidence that the cardiac stimulant

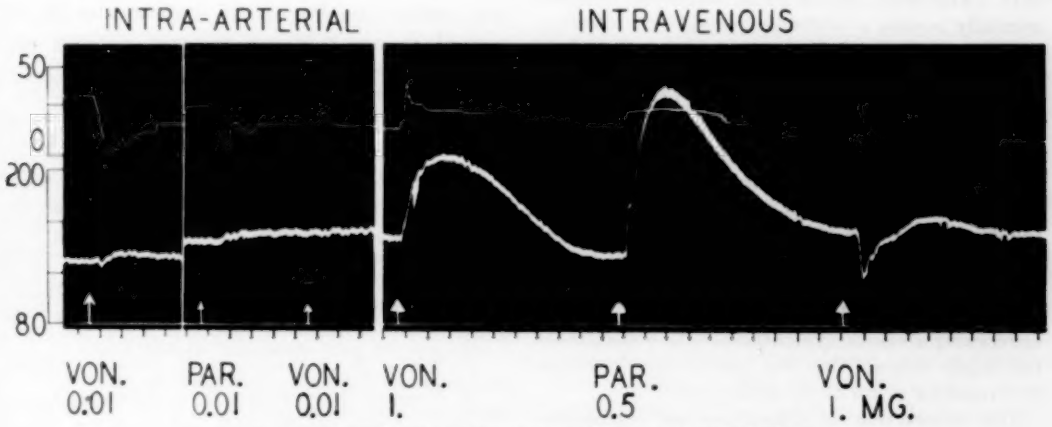

Fig. 1.—Carotid Blood Pressure and Femoral Blood Flow.

Dog, male, 13 Kg., Upper: Blood flow in cc. per minute. Center: Mean arterial blood pressure in mm. of Hg. Lower: Base line (at 80 mm. Hg) and time signals at one-minute intervals. At the first break in the record fifteen minutes and at the second break sixty minutes have been deleted. For the intra-arterial administrations the compounds were injected in a volume of 0.1 cc. into the peripheral side of the flowmeter. Intravenous administrations were made into the femoral vein. Note the active vasodilation produced by the Vonedrine after Paredrine. This dilation is shown by the increased blood flow without any change in arterial pressure after intra-arterial injection. On intravenous administration the dilation is more apparent as the flow increases during the marked fall in pressure. The increased blood flow which occurs with the first intravenous doses of Vonedrine and Paredrine is a passive increase due to the high arterial pressure.

RESULTS AND DISCUSSION

The observation that Paredrine reversed the pressor action of Vonedrine was first made during the course of another study on the sympathomimetic amines. It was observed that doses of 1 to 3 mg. of Vonedrine lowered the blood pressure in an animal which had received 1 mg. of Paredrine twenty minutes before. This observation was then confirmed in several other animals. Figure 1 shows the characteristics of this reversal effect, Vonedrine action of Vonedrine is inhibited by the Paredrine. The effect of Vonedrine after Paredrine probably represents the algebraic sum of the diminished cardiac stimulation and the active peripheral vasodilation. When equal doses of the two compounds are used the cardiac stimulant action of the Paredrine is completely inhibited, therefore only a depressor response due to the vasodilation appears. When the dose of Paredrine is less than the dose of Vonedrine the depressor phase is followed by a slight pressor response due to only partial inhibition of the

Vonedrine cardiac stimulation by the Paredrine.

A number of other amines were tested as to their influence on Vonedrine. These included tyramine (β-(*p*-hydroxyphenyl) ethylamine), Sympatol (β-(*p*-hydroxyphenyl) ethanolmethylamine), Propadrine (β-phenyl isopropanolamine), ephedrine, and epinephrine. None of these compounds inhibited or reversed Vonedrine and none of them restored the Vonedrine pressor action after Paredrine. Paredrine had no reversing action on any of these compounds. The Paredrine pressor action was unchanged following Vonedrine.

An inversion of the pressor action of the sympathomimetic amines may occur if the pressor response is removed by an antipressor agent, and if a normally masked depressor effect is present. Epinephrine reversal by the adrenolytic agents is due to this type of action. Benzylimidazoline hydrochloride (Priscol-Ciba), a sympathomimetic antipressor agent, has been shown to selectively block the pressor action of the amines without influencing their depressor action (11). Priscol effectively blocks the pressor action of Vonedrine; however, it does not produce Vonedrine reversal (11). This would indicate that Vonedrine does not normally possess a depressor action.

Some of the aliphatic amines have a depressor action which becomes apparent only after their pressor action is diminished through repeated doses or through the previous administration of ephedrine (3). Priscol does not bring out this depressor action of the aliphatic amines, but if the depressor action is present Priscol does not influence it (11). After the depressor action of Vonedrine had been elicited by Paredrine, administration of Priscol appeared to prevent the fall in pressure produced by the Vonedrine. Since Priscol has been shown to have no blocking action on sympathomimetic vasodilation this might indicate that the vasodilation produced by Vonedrine is not of the usual type.

This interaction of Paredrine and Vonedrine illustrates one of the chief difficulties in comparative studies on the sympathomimetic amines. The fact that one amine may greatly modify the response of another, which has often been overlooked or ignored, has given rise to some of the very divergent results reported in the literature. In comparing the activities of the sympathomimetic amines probably not more than one dose of the long-acting drugs should be used in any one animal in any one day. Even some of the so-called short-acting amines have actions which persist much longer than is shown by measurements of the mean blood pressure. This has been demonstrated by Kleinberg, *et al.* (12), who showed that epinephrine increases the intramuscular pressure for periods exceeding two hours.

SUMMARY

1. Paredrine reverses the pressor action of Vonedrine, changing its vasoconstrictor action to a vasodilator effect.

2. Epinephrine, ephedrine, Propadrine, Sympatol, and tyramine do not reverse Vonedrine and neither are any of them reversed by Paradrine.

3. This inversion of the Vonedrine pressor action by Paredrine appears to be an actual reversal of action and not an unmasking of a normally present response.

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Book Reviews

Acetanilid—A Critical Bibliographic Review, by MARTIN GROSS. Hillhouse Press, New Haven, Conn., 1946. 155 pp. 15 x 23.5 cm. Price $3.00.

This critique of acetanilid is volume one of a proposed series of monographs to be published by the Institute for the Study of Analgesic and Sedative Drugs. The topics discussed in this monograph are the history, physico-chemical properties, metabolism, therapeutic uses, pharmacology, toxicology, tolerance, habituation and addiction, and other factors pertaining to acetanilid medication.

The author evidently made a quantitatively thorough literature search as evidenced by the 763 references cited. It is the "critical" feature of the bibliography with which many will take issue. Most of the discussion centers around the toxic and undesirable properties of acetanilid. It is distinctly noticeable that the author is highly critical of papers showing the drug to be toxic while papers reflecting the opposite viewpoint are glossed over. A good example of this is the author's reaction to a paper by Kebler (cf. pp. 58–60). Kebler sent a questionnaire to 925 physicians and the 400 replying indicated 17 deaths attributable to acetanilid. The author's own survey covering the period showed 18 deaths from acetanilid and yet the author used nearly 3 pages to try to explain away Kebler's findings. To the reviewer, if there were 17 deaths resulting from acetanilid during the period 1886 to 1907, acetanilid is not a completely safe drug, even if the statistical approach of Kebler were not entirely sound.

The reviewer takes strong issue with the statement on page 59: "Opinions on the safety of a drug may be based on animal experiments, on the frequency with which toxic symptoms are observed in man, or on the magnitude of the difference between the lethal and therapeutic doses; *but the most compelling evidence is the record of destruction of human life by the drug*" (italics the reviewer's). The additional statement by the author that "there is no assurance that all or any large proportion of fatalities are reported" is not only an understatement but makes the preceding statement even more amazing. Certainly with a drug of the character of acetanilid, it is the daily exposure that is the important element. The reviewer is of the opinion that there are many cases of chronic acetanilid "poisoning" in which the general health of the person is improved by the withdrawal of acetanilid and yet the cases are not recorded in the literature.

The completeness of the documentation and the evidence of needed careful studies by more modern methods make the book worth while if it is put to genuine scientific purposes and not used as an apology.—MELVIN W. GREEN.

Richter's Chemistry of the Carbon Compounds, Volume III. Edited by RICHARD ANCHÜTZ and translated by A. J. MEE. Elsevier Publishing Company, New York, 1946. xviii + 794 pp. 13.5 x 21.5 cm. Price $15.00.

Volume III of *Richter*, which is concerned with the aromatic compounds, is so well known that it scarcely needs a review. Organic chemists are grateful to the publisher for making available this volume in spite of war conditions. Particularly helpful are the references to the original literature rather than *Chemisches Zentralblatt*.

Pharmacists will be disappointed, however, in the poverty of information on recent medicinal agents. Sulfanilamide is the only sulfonamide mentioned and it rates about one-third of a page. Nothing is said concerning the chemistry of the multiplicity of biochemical agents having a steroid nucleus. Procaine is barely mentioned and ephedrine is the only sympathomimetic drug considered.

In spite of pharmaceutical shortcomings, Richter will continue to fill a valuable place on the reference shelves of all interested in the many facets of organic chemistry—MELVIN W. GREEN.

Physical Methods of Organic Chemistry. Volume II. Edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., New York, 1946. vii + 629 pp. 23 x 14.5 cm. Price $8.50.

Volume II of this reference book contains chapters on: Spectroscopy and spectrophotometry; colorimetry, photometric analysis, and fluorometry; polarimetry; determination of dipole moments; conductometry; potentiometry; polarography; determination of magnetic susceptibility; determination of radioactivity; and mass spectrometry.

The subjects covered by this volume are so diversified that it is difficult to evaluate the book as a whole. One of the basic impressions that the reader obtains is that there is too much emphasis on instrumentation and not enough application to organic chemistry.

The sections on spectroscopy and spectrophotometry are very helpful. One questions the value of a separate chapter on photometry since there is considerable duplication with the spectroscopy chapter. The portions dealing with infrared spectroscopy and the Raman effect are much too short.

Most of the description of optical devices for polarimetric measurements are generally available. The section on nonvisual and spectropolarimetry are unusually good, however.

The chapter on conductometric titrations includes both a section on conductometric titrations and a

section on electrophoresis. In Dr. Michaelis' chapter on potentiometry, potentiometric titrations, *p*H, and oxidation-reduction potentials are described.

Dr. Müller's chapter on polarography is replete with examples of application to organic chemistry. Not only the qualitative and quantitative aspects are described, but the author gives considerable attention to the study of tautomerism, polymerization, and related phenomena.

The section on radioactivity contains no application, but is limited to methodology. The section on mass spectroscopy gives considerable valuable information to the organic chemist. The techniques are too cumbersome and costly for the average laboratory, however.

The two volumes of this excellent reference book should open new vistas to the organic chemist. The physical-chemical approach to organic chemistry is throwing new light on many old and new organic problems and *Physical Methods of Organic Chemistry* will prove to be a helpful guide to this rapidly expanding field.

For a review of Volume I of this book the reader is referred to THIS JOURNAL, **35**, 32(1946).—MELVIN W. GREEN.

Introduction to Emulsions, by GEORGE M. SUTHEIM. Chemical Publishing Company, Brooklyn, 1946. vii + 260 pp. 10.5 x 17 cm. 22 illus. Price $4.75.

The book is divided into six chapters dealing with the theoretical foundation, physical chemistry and chemistry of emulsifying agents, and formation, properties, and applications of emulsions. The basic considerations, underlying principles, and theories are well presented, and offer a good foundation for one who is interested in the fundamentals of emulsification.

The background is less pharmaceutical than commercial or technical, little actual consideration being given to the operation of preparing extemporaneous emulsions as practiced by the pharmacist of today. No mention is made of the use of Pharmagel (Gelatin) as developed by Tice, nor is there any reference or illustration to the very popular and efficient hand homogenizer.

The book contains an extensive table of emulsifying agents and pertinent facts relating to them. These agents are primarily of the newer type commonly referred to as wetting agents, mostly of commercial and industrial interest. A comprehensive and valuable bibliography is also included.

The book is a worthy addition to the library of anyone interested in the principles of emulsification. —ADLEY B. NICHOLS.

Organic Qualitative Microanalysis, by FRANK SCHNEIDER. John Wiley and Sons, New York, 1946. iv + 218 pp. 136 figs. 13.5 x 21.5 cm. Price $3.50.

Microchemistry, now a recognized specialty in the chemical field, has progressed very rapidly during the past decade. Qualitative organic microchemistry has been the most backward in the microfield, while quantitative organic microchemistry has probably progressed the furthest.

Professor Schneider has written this book to be used as a textbook and laboratory manual by organic chemistry students interested in the systematic identification of organic compounds. The system follows very closely the Mulliken–Huntress classification, but aims to use a sample about $^1/_{100}$ the normal size.

Methods of purifying samples containing mixtures, determining physical constants of pure organic compounds, and preparing characteristic identifying derivatives are given. Most tests and techniques are described in considerable detail and with adequate diagrams to facilitate the work.

In the hands of an instructor familiar with microprocedure, this book no doubt will serve as an excellent guide to a fascinating field. The novice and research worker, using such testing only occasionally, will probably find the fact that such careful attention must be given to details of the technique a source of discouragement.—MELVIN W. GREEN.

Colloids—Their Properties and Applications, by A. G. WARD. Interscience Publishers, Incorporated, New York, 1946. 133 pp. 11 x 18 cm. Price $1.75.

This little volume is not intended to be a complete treatise on the colloidal state, but rather to review briefly colloidal chemistry and its modern application in simple, quasi-technical language.

The book is divided into three parts with a total of 17 chapters. Part I deals with the nature of the colloidal state and discusses atomic structure, the physics of surfaces and the physical chemistry of the colloidal state.

Part II deals with colloidal systems. Here are discussed very succinctly the lyophobic and lyophilic systems as well as gels, pastes, emulsions, foams, dusts, smokes, and fogs.

The third part is concerned with the application of colloidal theory to rubber, cellulose technology, proteins, clay, paints and varnishes, detergency, and to life processes.

While not adequate as a textbook in a course in colloidal chemistry at the university level, this little book is fascinating to read and well worth while as a quick source of review.—MELVIN W. GREEN.